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# PROGRESSIVE OXIDATION OF COLD-STORAGE BUTTER

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## OUTLINE OF PREVIOUS WORK

Much has been written concerning the changes occurring in butter. The word "change" is here used in its broad and general sense to include any perceptible alteration whatsoever, although it refers principally to an organoleptic one, whether induced by one or several factors.

Butter has been kept for certain periods of time during which it has been exposed to the action of various decomposing and disintegrating agencies, and a study of the products of change thereby resulting has led investigators to draw conclusions relative to the causation of the "off flavors" so often found in stored butter. As a general rule, the majority of opinions advanced in accounting for the deterioration of butter seem to have been based either upon insufficient analytical data or upon a study of butter or butter fat kept under conditions which prevail only to a very limited degree when butter is stored.

Many investigators confined their attention to a study of the fat of butter alone and sought to attribute the appearance of undesirable flavors in whole butter to some change which this one constituent undergoes. However, more recent investigations carried on with fats other than butter would appear to render such an assumption doubtful and would seem to make imperative more conclusive information concerning the causation of disagreeable flavors in whole butter held in cold storage.

The early literature in regard to the chemical changes which take place in butter is voluminous, but it is also conflicting and confusing, a great deal of it being of a purely speculative nature.

A great variety of bodies, products of chemical change, have presumably been identified in butter kept under varying conditions. The confirmation of the presence after a certain interval of time of such substances in fats known to have been originally pure is of value; yet such data obtained in the investigation of a material containing other constituents as well are obviously not so satisfactory unless it is definitely known that these attendant components do not likewise undergo

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similar changes. Acids, aldehydes, alcohols, and esters, among other things, may have been identified in spoiled fats, and even up to the present time it has been customary to attribute their origin solely to the fat itself. The reason for such deduction is evident. It is well known that the fat of butter is in itself a most complex material. It is a composite, made up of mixtures of the glycerids of fatty acids. Among the saturated glycerids butyrin is an essential ingredient, although palmitin and myristin predominate. Olein has generally been considered to be the only unsaturated glycerid in butter fat, yet quite recently Laxa and Konecny (3)[1] claim to have found that the fatty acids of the "liquid fat" of separator slime consist of 49.65 per cent of erucic acid and 21.24 per cent of oleic acids; but this assumption may not be entirely justified.

The improbability of any chemical change occurring in the saturated glycerids of storage butter is quite generally recognized; consequently the glycerid olein, purely because it contains an unsaturated linkage in the molecule and absorbs the halogens with avidity, has been considered as the source from which are derived those decomposition products the presence of which in fats influences their more or less decreased value. As a matter of fact, any satisfactory and conclusive evidence that the olein of butter fat is readily susceptible to oxidation under conditions similar to those prevailing when butter is stored is entirely lacking. On the other hand, it has been demonstrated that pure olive oil, the liquid glycerids of which consist almost entirely of olein, shows very little absorption of oxygen as measured by the iodin number, even after having been kept for three years under ordinary conditions (5). Masters and Smith (6), in preliminary experiments with butter fat, found but little change in the iodin value during cooking experiments carried out with this material. To obtain any pronounced change in the iodin value and in the acidity, they found it necessary to heat their samples of butter fat to as high a temperature as 200° C. while passing oxygen through the material, the mere heating of the fat to such temperature under ordinary conditions proving to be insufficient. From these two illustrations, as well as from more recent work done by other investigators, the discussion of which owing to limited space is omitted, it must be concluded that the possibility of the olein of butter fat undergoing an appreciable oxidation caused by the small quantity of atmospheric air inclosed in a package of butter is very remote, especially when it is remembered that butter is stored in the dark at a temperature considerably lower than the freezing point of water.

The inability of chemists to judge the quality of an edible fat because of the absence of satisfactory chemical data has been frequently pointed out, and this is attributable primarily to the lack of appropriate and comprehensive analytical procedure. For instance, rancidity has generally been regarded as the natural concomitant of acidity, yet a pronounced

[1] Reference is made by number to "Literature cited," p. 951.

rancidity may have appeared in a stored fat without the manifestation of any increased acidity as measured by a simple titration. Again, an undue significance may be attached to a slight decrease in the original iodin number of the fat. Such a decrease is usually considered to be caused by the taking up of oxygen by the double bond of the unsaturated glycerid; yet it must be remembered that self-polymerization—the interlocking of two or more molecules of the unsaturated glycerids—may occur, a condition which would likewise bring about a lowering of the iodin value. Again, the olein of butter fat may not exist entirely as the normal glycerid, and it is possible that a certain amount of this glycerid may occur as an isomerid. So far as is known to the writer no work has been carried out to determine whether the olein of stored butter is present entirely as the normal glycerid. In this connection it may be observed that the work of Ponzio and Gastaldi (8) and of Fokina (1) indicates that the farther the double bond is removed from the carboxyl group the nearer the iodin number approaches the theoretical value. Normal oleic acid gave the theoretical value of 90. On the contrary, 2–3 oleic acid gave a Hübl number of only 6.6, Wijs 20.4, Hanus 1.9. While there are no data at hand at present to prove that 2–3 oleic acid actually does occur in butter fat, yet this contingency is quite possible; and it is well to take it into consideration as yet another factor which may produce a slight lowering of the iodin number of stored butter. It is evident, however, that the customary methods in vogue to determine the quality of fat leave much to be desired.

One of the factors so often construed as influencing the appearance of undesirable flavors in a fat is the nature of the impurity, or impurities, contained therein. In just what manner these foreign substances bring about these undesirable characteristics has not been fully cleared up, because it is conceivable that it depends upon several parallelly progressing chemical reactions and because it is possible that slight chemical changes really difficult of identification by analytical methods suffice to produce the above-mentioned disagreeable features.

It is apparent that even at the present time there seems to be considerable doubt as to whether the undesirable flavors of storage butter arise from a decomposition occurring in the fat itself or in some one or more of the other components entering into the composition of the whole product. For this reason it is thought advisable to confine the preliminary work on this subject to an attempt to settle this most basic consideration before proceeding with the further investigation of the causation of the "off flavors" so frequently met with in storage butter.

## STATEMENT OF THE PROBLEM AND METHOD OF SOLUTION

Even in those times when the chemical constitution of the fats was still unknown it had been surmised that the changes which oils and fats underwent on keeping were simply the result of oxidation. This is the

view most generally held at the present time, and the more recent literature on the subject indicates that this phase of research is to be continued with no less abated interest. It is still unknown whether the development of undesirable flavors in storage butter is dependent upon an oxidation occurring in the fat itself or whether the milk sugar and nitrogenous constituents of the curd are those components of the butter most susceptible to oxidation. Approximately 10 per cent of the volume of butter is air (9), and it is quite possible that, owing to the oxygen of the air inclosed within the material, a slight and progressive oxidation may take place in the interior of a package of butter. This possibility, when considered together with the known fact that marked and undesirable alterations in the flavor of butter during storage may be brought about by acidifying the pasteurized cream from which the butter is made (10), has suggested the idea that an examination of the air inclosed within packages of butter differently prepared and in butter fat alone might furnish some interesting data as to whether the undesirable chemical changes occurring in stored butter are caused by a progressive oxidation in the fat itself or in some one or more of the nonfatty ingredients.

It was deemed advisable to pursue this line of investigation in a manner not previously attempted, so far as known. Samples of pasteurized sweet-cream butter, butter made from pasteurized cream to which lactic acid had been added, and butter made from pasteurized cream to which a starter had been added and which was churned at once, were prepared, packed in glass tubes, and stored. Tubes from each lot were removed from storage after certain intervals of time had elapsed and an analysis of the air therefrom was made by means of the gas apparatus specially designed for the purpose. (See fig. 1 and Pl. CXI.) It was hoped that the analytical data so obtained would show some distinguishing features between the three samples dissimilarly prepared, especially with respect to the sample made from acid cream. It was also decided to make use of the determination of the chemical constants of the pure butter fat to serve merely as an indication as to whether any chemical alteration of the fat through oxidation had occurred during the storage interval, confirmed by the analysis of the air extracted from packages of butter fat to determine whether the oxygen content therein is diminished during the storage period. The data so obtained were used as a standard, and the aim kept in view was to study the effect, if any, of the presence of varying amounts of nonfatty constituents (protein, lactose, etc.) upon the decomposition of the fat of butter and, in addition, to note whether the presence of varying quantities of these substances in the butter induced an alteration during storage in the composition of the air incorporated in the samples at the time of their manufacture. Samples of pure butter fat and of butter containing varying quantities of buttermilk

were also prepared, packed into tubes, and stored under the same conditions as the foregoing samples. The effect of a large amount of air upon a small quantity of butter fat and upon buttermilk containing varying quantities of acid was studied by filling other tubes with pumice fragments which were then impregnated with fat or buttermilk and an analysis of the air therefrom made after certain intervals in storage had elapsed.

## DESCRIPTION AND MANIPULATION OF THE GAS APPARATUS USED

In figure 1 is depicted the apparatus constructed for use in the extraction and analysis of the air confined in the packages of the various

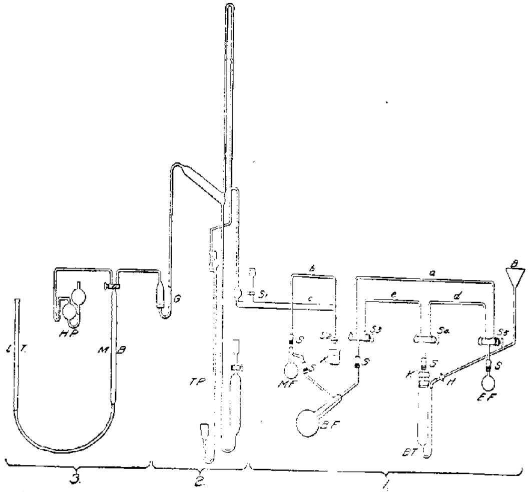


FIG. 1.—Diagram of gas apparatus used in the extraction and analysis of the air confined in butter.

samples of butter fat and butter put up and stored for the investigation which has been described. The apparatus is of glass throughout and consists of three divisions: (1) The system for extracting the gas from the butter tubes, (2) the Töpler pump for transferring the gas so obtained to (3) the usual Hempel apparatus. The rigid and undetachable arrangement of glass tubing and mercury-seal stopcocks comprising the upper part of 1 is conveniently fastened to a wooden frame by means of small, brass pipe bands in the manner seen in Plate CXI, which shows the entire apparatus set up for use. The lower, detachable parts of the extracting system (see fig. 1) consist of the butter tube B. T., the construction and nature of which are described later and which contains the sample

under investigation; the butter flask B. F., of about 1 liter capacity, for retaining the sample after its passage through part of the system; the moisture flask M. F., of 200 c. c. capacity, for retaining the greater part of the moisture liberated from the sample; and a small evacuation flask or globe, E. F., of about the same capacity. These detachable parts are connected with the upper part of the system by means of various mercury seals, S, S.

The operation is begun late in the afternoon of the day before the actual determination by putting the entire division 1 under vacuum and allowing it to stand in this condition overnight. This is done in the following manner:

With the exception of the butter tube B. T., the apparatus is connected as illustrated: The mercury-seal stopcocks $S_1$, $S_2$, and $S_4$ are closed and stopcocks $S_3$ and $S_5$ are so turned as to open the system from E. F. through *a*, B. F., M. F., *b*, and *c* to the Töpler pump T. P. The Töpler pump is now given one stroke, which serves somewhat to exhaust the air confined in the system; a small beaker, *x*, filled with concentrated sulphuric acid is brought under stopcock $S_2$ so that the tube projecting downward from the stopcock is plunged well beneath the surface of the acid, and the beaker is supported in this position. Stopcock $S_2$ is now cautiously opened until the acid rises to form a long level of drying agent covering the bottom of tube *c*, when the influx of acid is stopped. The pump is now worked to its limit of exhaustion (about 0.3 mm. on the McLeod gauge). A turning back and forth of stopcock $S_5$ accompanied with successive strokes of the pump will evacuate tube *d* to stopcock $S_4$, and this is followed by turning stopcock $S_3$ and working the pump to exhaust the tube *e*. The entire division 1 is now under exhaust and is allowed to remain so for a considerable length of time, preferably overnight. The next morning, if the gauge indicates that no leakage of air into the system has occurred, the actual determination is made as follows:

The moisture flask M. F. is covered by a beaker which is then packed with cracked ice and salt (sodium chlorid). The butter tube B. T. is connected at $S_4$ by means of a mercury seal. Funnel B is closed at stopcock H, and filled with a three-fourths saturated sodium-chlorid solution at a temperature of 50° C. A little of this brine solution is allowed to trickle from a pipette into the small side tube of the butter tube B. T. until the latter is completely filled, whereupon it is connected with the funnel tube below H by means of a piece of tight-fitting rubber vacuum tubing. A large glass jar (not shown in the illustration) is now used to cover the butter tube B. T., the base of which rests upon a large rubber stopper with its center removed. The mercury-seal stopcock $S_5$ is now turned to connect the evacuation flask E. F. with *d*, and a turn of $S_4$ toward *d* followed by a closing of the same serves to evacuate the tube from $S_4$ to the glass stopcock K of the butter tube B. T. $S_3$ is opened to connect *e*

with the system B. F., etc. The stopcocks $S_4$, K, and H are now all closed. Water at a temperature of 45° C is poured into the glass jar surrounding B. T. until it immerses the rubber stopper carrying the stopcock K and the rubber connection between the small side tube to B. T. and H. The warmth thus applied to the butter tube at once causes a slight pressure against K and H. H is opened first to allow one or two trapped bubbles of air to escape up toward B and is then closed. K is immediately opened and is soon after followed by the opening of H again. As the material in B. T. melts, a graduated and regulated opening of $S_4$ permits most of the air confined within the sample to pass over into the system, and the remaining air follows with the melted fat, etc., which passes up, around, and down through *e* and trickles into B. F. A too rapid passing of butter containing much curd should be prevented, as it will cause considerable foaming in the butter flask. The warm salt solution flowing in from B displaces the sample from B. T. When the material has been thus removed from B. T. and the level of the salt solution has reached $S_4$, this stopcock is closed, followed also by the closing of $S_3$. The gas is now transferred from the apparatus by the Töpler pump to the gas-collecting tube G, allowing a few minutes to elapse between strokes of the pump, thus permitting the gas containing moisture not removed by M. F. to become dried by passing over or remaining in contact with the sulphuric acid in *c*. The gas is collected over mercury in G and is drawn therefrom into the mercury-filled measuring burette M. B. connected with the leveling tube L. T., from which it is passed over into the Hempel pipettes H. P., where the quantities of carbon dioxid and oxygen in the gas are determined in the usual manner with solutions of potassium hydroxid and alkaline pyrogallol.

## SPECIAL BUTTER TUBES[1]

These tubes are about 9 inches long and 1¾ inches in diameter, with necks widened somewhat to accommodate a No. 9 rubber stopper carrying a glass stopcock. An ordinary-sized glass tube, bent on itself, leads upward from the base. Each of these tubes when packed will contain about 250 gm. of butter.

These tubes were cleaned, sterilized, and packed with the sample, allowing a very small air space between the surface of the butter and the rubber stopper. Pure, neutral, paraffin oil was poured on the surface of the butter and the stopper was pressed in until the oil in the tube had risen above the stopcock. The stopper was wired down tightly and the stopcock closed. A few cubic centimeters of paraffin oil were then allowed to flow down the side tube. Butter packed in this manner is free from contact with the outside air.

[1] The use of these tubes for packing and storing butter was suggested by Mr. L. A. Rogers, of the Dairy Division.

## EFFECT OF CREAM ACIDITY UPON THE COMPOSITION OF THE AIR IN BUTTER HELD IN STORAGE

The samples, the gas-analysis data of which are given in Tables I, II, and III, were prepared under conditions as nearly identical as possible, the butter having been made at Troy, Pa. In each case the butter was made from 60 pounds of cream taken from one lot, pasteurized at 140° F., and cooled to 48° F. In all three cases the temperature of the buttermilk was 58° F., the quantity of salt added to the butter each time was 12 ounces, and each working was carried to 15 revolutions.

The cream of sample 1 was churned sweet. To the cream of sample 2 was added 15 per cent of the starter, and the churning done at once. Before churning the cream of sample 3, sufficient lactic acid was added to it to make its acidity 0.71 per cent (calculated as lactic acid) by titration.

TABLE I.—*Analysis of air extracted from sweet-cream butter*

[Calculated to 0° C. and 760 mm. Acidity of cream as lactic acid, 0.11 per cent; salt, 1.21 per cent; curd, 0.58 per cent]

| Number of bacteria per gram.[a] | Time stored. | | | Oxygen. | Carbon dioxid. |
|---|---|---|---|---|---|
| | At 0° F. | At 32° F. | At room temperature. | | |
| | *Days.* | *Days.* | *Hours.* | *Per cent.* | *Per cent.* |
| 9,050,000 | 0 | 0 | 0 | [b] 25.15 | [b] 2.89 |
| | 0 | 2½ | 1 | 22.23 | 4.51 |
| | 0 | 15 | 1 | 15.96 | 7.58 |
| | 0 | 41 | 1 | 9.86 | 11.91 |
| | 0 | 57 | 1 | 5.49 | 15.24 |
| | 81 | 0 | 1 | 25.51 | 1.49 |
| | 81 | 1 | 1 | 22.70 | 2.02 |
| 132,000 | 81 | 13 | 1 | 20.45 | 2.86 |
| | 110 | 0 | 5 | 20.62 | 2.85 |
| | 110 | 1 | 1 | 23.00 | 2.73 |
| | 150 | 0 | 2 | 24.18 | 1.62 |
| | 180 | 0 | 1 | 25.11 | 0.57 |

[a] Thanks are due Mr. L. A. Rogers, of the Dairy Division, for the bacteriological work in connection with this investigation.
[b] Analysis of gas extracted from butter as soon as tube was packed.

Samples 1 and 2 were shipped on the afternoon of the same day, arriving in Washington, D. C., shortly before noon of the following day, when the butter was immediately packed into sterilized special glass tubes and small jars and then placed in storage at 0° F. Sample 3 was finished late in the afternoon of the same day on which the preceding samples were made, and did not reach Washington until the second morning after, when the butter was at once packed into tubes and jars and placed in storage under the same conditions as above.

From each of the three samples several tubes were taken and transferred to storage at 32° F. The remainder of the tubes were allowed to continue at a storage temperature of 0° F. From time to time tubes were removed from both temperatures, the gas removed therefrom by

means of the specially devised apparatus, and the quantities of carbon dioxid and oxygen determined.

A perusal of Table I discloses the fact that very little alteration occurred in the composition of the air inclosed in this sample of sweet-cream butter made from cream having an acidity of 0.11 per cent when it was kept for about 6 months at a temperature of 0° F. During this interval practically no diminution of the original oxygen content took place, and the only apparent change to be noted is a probable decrease in the small quantity of carbon dioxid which was known to be present in the butter at the time it was made. An appreciable and progressive change did occur, however, when the butter was kept for nearly two months at a temperature of 32° F. In this case it will be noted that the original oxygen content decreased, while there was a corresponding increase in the initial quantity of carbon dioxid.

Every effort was made to keep the tubes containing this sample, as well as those containing the other differently prepared samples, under comparable conditions. In this connection it may be mentioned that, since it is necessary to surround the tubes with warm water (45° C.) to melt the butter sufficiently to cause it to flow through the apparatus used and that this procedure if carried out immediately upon the removal of the tubes from storage might result in cracking them, the plan was adopted of allowing them to warm up slightly at room temperature for one hour, except in two cases, in which the tubes were intentionally permitted to remain a longer period at room temperature for the purpose of obtaining additional information. It was found that a tube of this butter, when allowed to remain for 5 hours at room temperature after a storage period of 110 days at a temperature of 0° F., contained less oxygen than a corresponding tube of the same sample kept for the same length of time at a temperature of 0° F., and for 1 day at a temperature of 32° F. In measuring the effect of raising the storage temperature to 32° F. on a sample which had been stored at 0° F. for 81 days, it is of interest to note that after holding for 1 day at the higher temperature there is a measurable decrease in the quantity of oxygen known to be present in the sample after the 81 days at the lower temperature, and this effect on the same sample is much more pronounced after holding at the higher temperature for an additional 12 days, or a total of 13 days.

It may be concluded, therefore, that sweet-cream butter prepared as this sample was and containing a considerable number of bacteria will show but little alteration in the composition of the air inclosed in it when it is kept for six months at a temperature of 0° F. A perceptible change, however, occurs when the butter is kept at a temperature of 32° F., and a very noticeable one when it is kept at room temperature. The sample of butter used scored 92 when made and 91 at the end of three months. After a period of six months in storage at a temperature of 0° F. the score was given at 90, there being no trace of any undesirable flavor.

TABLE II.—*Analysis of air extracted from butter made from sweet cream churned immediately after the addition of 15 per cent of a commercial starter*

[Calculated to 0° C. and 760 mm. Acidity of cream as lactic acid, 0.25 per cent; salt, 1.19 per cent; curd, 0.59 per cent]

| Number of bacteria per gram. | Time stored. | | | Oxygen. | Carbon dioxid. |
|---|---|---|---|---|---|
| | At 0° F. | At 32° F. | At room temperature. | | |
| | *Days.* | *Days.* | *Hours.* | *Per cent.* | *Per cent.* |
| 680,000 | 0 | 7 | 1 | 10.89 | 26.44 |
| | 40 | 0 | 1 | 11.64 | 25.35 |
| | 55 | 0 | 1 | 10.84 | 22.34 |
| 160,000 | 80 | 0 | 1 | 10.70 | 21.87 |
| | 100 | 1 | 1 | 10.95 | 20.92 |
| | 150 | 15 | 1 | 8.78 | 19.27 |
| | 205 | 0 | 1 | 9.00 | 19.30 |

After the addition of a starter the acidity of the cream from which the butter of sample 2 was made was a little more than twice that of the cream used in the preparation of the sample of sweet-cream butter. A slight but appreciable decrease in the oxygen content of the sample during storage at a temperature of 0° F. was observed, while a perceptible decrease in the carbon dioxid was also manifested. After the sample had been kept for a period at a temperature of 0° F., the effect upon the composition of the air in the butter after standing for several days at a temperature of 32° F. was tabulated, as shown in Table II. This table also shows that a sample of butter made in this manner displays, so far as the composition of the air inclosed in it is concerned, a comparatively slight variation from that observed in the previous case of sweet-cream butter, when both samples are stored at a temperature of 0° F. This sample of butter scored 92 when made, 90 after three months' storage at a temperature of 0° F., and 89 after six and one-half months' storage at the same temperature, there being practically no variation in the flavor during the interval.

The addition of lactic acid to the cream of butter sample 3 before churning brought the total acidity to nearly three times that of the cream used to prepare the foregoing butter of sample 2, and about six and one-half times that of the cream used in making the sweet-cream butter. A pronounced decrease, greater than that observed in either of the two previously given samples of butter, occurred in the oxygen and carbon-dioxid content, even when the butter was stored at a temperature of 0° F., and this decrease was still more marked when it was allowed to remain at a temperature of 32° F. The score of this butter, originally 93, fell to 88 after three months in storage at a temperature of 0° F., and at the end of this interval it had an unclean flavor which was still more pronounced after a period of six months' storage at the same temperature, when the score was 84.

TABLE III.—*Analysis of air extracted from butter made from sweet cream churned immediately after the addition of lactic acid*

[Calculated to 0° C. and 760 mm. Acidity of cream as lactic acid, 0.71 per cent; salt, 0.85 per cent; curd, 0.55 per cent]

| Number of bacteria per gram. | Time stored. At 0° F. | Time stored. At 32° F. | Time stored. At room temperature. | Oxygen. | Carbon dioxid. |
|---|---|---|---|---|---|
| | *Days.* | *Days.* | *Hours.* | *Per cent.* | *Per cent.* |
| 2,050 | 0 | 6 | 1 | 21.58 | 11.20 |
| 0 | 0 | 32 | 1 | 20.53 | 11.08 |
| 0 | 0 | 48 | 1 | 16.70 | 6.74 |
| 0 | 0 | 62 | 1 | 14.93 | 3.86 |
| 0 | 0 | 80 | 1 | 5.95 | 4.45 |
| 0 | 0 | 82 | 1 | 4.17 | 4.54 |
| 0 | 75 | 0 | 1 | 17.30 | 4.48 |
| 0 | 104 | 0 | 1 | 16.94 | 1.79 |
| 0 | 140 | 0 | 1 | 11.74 | 1.75 |
| 0 | 202 | 0 | 1 | 10.84 | 1.54 |

Having now determined that the decomposition caused by cream acidity progresses at a temperature of 0° F. in a package of butter and can be measured by an analysis of the gas extracted therefrom, the next step in the investigation of the problem concerning the development of "off flavors" in storage butter involved a series of experiments the purpose of which was to determine whether this measurable decomposition occurs in the fat of the butter itself, in the buttermilk, or in both.

## OXIDATION OF PURE BUTTER FAT [1]

The butter fat used in the following determinations was prepared to exclude, so far as possible, by melting, filtering, and washing all ingredients of the butter other than fat and was made from the same lot of cream as the samples of butter $B_1$ and $B_2$, mentioned later. The butter was warmed in a glass vessel to from 32° to 34° C. and allowed to stand, to separate the fat from the greater part of the nonfatty substances. The supernatant fat was then siphoned off, filtered into water at 12° to 14° C., and then thoroughly agitated to granulate it. The fat was then washed several times, salted, and worked on a table worker to the extent of 40 revolutions. The butter fat so prepared was found to contain but 0.05 per cent of protein (total N×6.38). It was packed in absolutely clean and sterile glass jars and also in the special glass tubes for air analy-

[1] The term "pure butter fat" is merely relative. Osborne and Mendel (7) have affirmed that butter fat prepared by centrifugalizing melted butter and pipetting off the clear fat was "entirely free from nitrogen and phosphorus and was devoid of any ash-yielding or water-soluble components." Funk and Macallum (2) have recently challenged this statement as regards nitrogen, since they find that butter fat prepared according to Osborne and Mendel's directions yields easily measured quantities of nitrogen in each of the repeated washings with dilute acid and they conclude that it is very difficult and perhaps impossible completely to free butter fat from nitrogenous substances. McCollum and Davis (4) state that their experiments with butter fat tend to strengthen the conclusion drawn by Funk and Macallum regarding the difficulty of completely freeing the butter fat from nitrogen.

sis. The fat in the jars was covered with a thin layer of paraffin to exclude any action of the atmosphere other than that contained within the material itself. All samples were kept under the same conditions in cold storage at a temperature of 0° F. Samples taken from the lot packed in jars were at once analyzed and, in addition, were scored by Messrs. Corneliuson and Rabild, of the Dairy Division. After intervals of approximately one month, samples were withdrawn from storage, analyzed, and scored. This was continued for several months, during which time a sufficient period had elapsed for the samples to manifest any change which might occur in butter stored for a reasonable length of time.

As may be seen by reference to Table IV, it is certain that no alteration in this sample of butter fat was manifested by the flavor. These samples of nearly pure butter fat showed no physical alteration of any kind after six months or even after one and one-half years in cold storage. There was no development of any characteristic flavor whatsoever, the scoring indicating what might have been expected in case of a material deprived of nearly all its essential ingredients other than fat.

TABLE IV.—*Scores of butter fat stored at 0° F.*

| Age. | Score. | Remarks. | Scorer. |
|---|---|---|---|
| *Months.* | | | |
| 1 | 88 | Oily, clean flavor | Corneliuson. |
| 2 | 87 | .....do | Do. |
| 3 | 87 | .....do | Rabild. |
| 4 | 87 | .....do | Do. |
| 5 | 87 | .....do | Do. |
| 6 | 87 | .....do | Do. |
| 18 | 87 | .....do | Do. |

As noted earlier in this paper, the following determinations were made to establish a standard as a criterion for judging any change which might occur in the fat of the same lot of butter (whole butter) prepared with varying quantities of nonfatty ingredients (Table V).

TABLE V.—*Chemical constants of the butter fat after being nearly freed from the nonfatty ingredients by melting, filtering, and washing and stored at 0° F.*[1]

| Age. | Reichert-Meissl number. | Iodin number. | Saponification number. | Soluble acids as butyric. | Insoluble acids. | Acetyl value. | Free acid as oleic. |
|---|---|---|---|---|---|---|---|
| | | | | *Per cent.* | *Per cent.* | | *Per cent.* |
| Initial | 30.03 | 37.30 | 226.8 | 5.552 | 87.54 | 3.703 | 0.456 |
| 2 months | 30.17 | 37.42 | 226.8 | 5.572 | 88.10 | 3.785 | .468 |
| 3 months | 29.84 | 36.58 | 226.9 | 5.483 | 87.52 | 3.634 | .427 |
| 4 months | 29.67 | 36.68 | 226.4 | 5.140 | 87.22 | 3.340 | .408 |

[1] The determinations of chemical constants of the fat incorporated were made by Dr. E. C. Thomssen, formerly of the Dairy Division.

These figures would seem to indicate that very little, if any, chemical change occurred in the fat after having been kept in storage at a temperature of 0° F. for a period of four months, and it was so apparent that no pronounced change could be expected until a longer time had elapsed than is usually practiced in storing butter that this experiment was discontinued. It was apparent from the analysis of the fat that no noteworthy oxidation had occurred therein while the experimental samples were held in storage. An analysis of the air confined within the butter fat is given in Table VI.

TABLE VI.—*Analysis of the air in butter fat, stored at 0°F., after being nearly freed from the nonfatty ingredients by melting, filtering, and washing*

[Protein, 0.05 per cent; total N X, 6.38; calculated to 0° C. and 760 mm.]

| Age. | Total gas. | Total carbon dioxid. | | Total oxygen. | | Calculated oxygen.[1] |
|---|---|---|---|---|---|---|
| *Months.* | *C. c.* | *C. c.* | *Per cent.* | *C. c.* | *Per cent.* | *C. c.* |
| 2 | 33.20 | 0.99 | 2.98 | 6.43 | 19.37 | 6.44 |
| 3 | 27.35 | .94 | 3.44 | 5.22 | 19.09 | 5.28 |
| 4 | 29.51 | .93 | 3.15 | 5.94 | 20.13 | 5.72 |
| 5 | 38.90 | 1.38 | 3.55 | 7.63 | 19.61 | 7.51 |
| 12 | 30.81 | 1.76 | 5.71 | 4.27 | 13.86 | 5.81 |
| 24 | 31.59 | .93 | 2.94 | .93 | 2.94 | 6.13 |

[1] After deducting the figure for carbon dioxid from total quantity of gas extracted from the tube, and assuming that the residual gas is pure air—that is, approximately one-fifth oxygen.

Practically all the carbon dioxid present in the gas extracted from these samples was evidently either in the butter fat at the time of its manufacture or was produced therein within a period of two months after being put into storage. Although the figures would seem to indicate a slight progressive increase in its amount during the storage interval, yet its total amount is small; and in view of the oxygen data obtained it seems to bear little or no relation to the oxygen content. It is very clear, however, that no appreciable oxidation of the nearly pure fat itself occurred during a storage interval of five months; and it was not until after the sample had remained in storage for one year that a slight, measurable oxidation was indicated. In this connection it is thought advisable to note the following general consideration:

Although the iodin numbers obtained for the first and second months and those obtained for the third and fourth months are so close as to resemble duplicate determinations, yet we will take it for granted that the total decrease in the iodin number during the entire period of the investigation is attributable exclusively to the absorption of oxygen by the olein of the fat and not to some one or more of the other factors which, as already indicated earlier in this paper, may influence the data obtained for the iodin number. If we regard 0.72 (the difference between 37.30 and 36.58) as representing the taking up of oxygen by the olein of the fat, the following calculations, based upon this hypothesis, will serve to point out the great improbability of any change in the fat from oxidation during storage at a temperature of 0° F.

Each tube containing the butter fat under investigation in the gas analysis held about 250 gm. of material, corresponding to about 200 gm. of pure fat.[1] The decrease in the quantity of iodin absorbed by a tube would be 1.44 gm., indicating that the fat had absorbed 0.091 gm., or 63.7 c. c. of oxygen. The total quantity of gas incorporated into the sample for the third month, for instance, was only 27.1 c. c., containing approximately but 5.28 c. c. of oxygen in all, and this is obtained from the tube in undiminished quantity in the gas analysis. After one year's storage the material had absorbed only 1.54 c. c. of oxygen, and even after two years' storage the presence of unabsorbed oxygen could still be determined. From the foregoing it will be seen that it is very improbable that any oxidation of pure butter fat occurs during storage at a temperature of 0° F. when the fat is stored for a reasonable length of time. It was decided, however, to make an additional experiment in order to be more certain on this point.

## BUTTER FAT EXPOSED TO A LARGE SURFACE OF AIR

A sample of butter fat was prepared in the same manner as was the preceding material—by melting, filtering, and washing. In addition, it was given a thorough agitation on the shaking machine with four successive changes of warm water containing 0.5 per cent of hydrochloric acid. The warm butter fat so prepared was allowed to flow through the side tube of the special butter tube filled with pumice fragments until it overflowed through the glass stopcock at the top. The tube was then inverted and the butter fat in the tube permitted to run out. In this manner a small quantity of fat, clinging to the pumice fragments, was exposed to the action of a large quantity of air, a condition just the reverse of that in the previous case. The tubes were then stored at 32° F., a temperature considerably higher than that used in the previous cases. The results are given in Table VII.

TABLE VII.—*Oxidation of pure butter fat exposed to the action of a large surface of air at 32° F.*

[Calculated to 0° C. and 760 mm.]

| Age. | Total gas. | Total carbon dioxid. | | Total oxygen. | | Calculated oxygen.[2] |
|---|---|---|---|---|---|---|
| *Days.* | *C. c.* | *C. c.* | *Per cent.* | *C. c.* | *Per cent.* | *C. c.* |
| 30 | 98.60 | 0 | 0 | 19.50 | 19.78 | 19.72 |
| 61 | 90.30 | 0 | 0 | 16.32 | 18.07 | 18.06 |
| 100 | 89.40 | 0 | 0 | 15.98 | 17.87 | 17.88 |

[1] Eight tubes of butter fat were put up for this investigation. The average weight of material in each tube was 249.5 gm. The butter-fat content of each tube was approximated as follows: This butter fat was prepared to represent normal butter minus the nonfatty constituents (protein, lactose, etc.). The effort was made to incorporate the average quantity of water into it, and salt also was added. With 1 per cent of salt and 16 per cent of water (the maximum) in the butter fat, 250 gm. of the material in the tube would consist of 2.5 gm. of salt, 40 gm. of water, and 207.5 of fat ("about 200 gm."). Taking any smaller percentage of water than 16 would increase the percentage of fat, which would, of course, call for a greater absorption of iodin than 1.44 gm., expressing the taking up of a greater quantity of oxygen than 63.7 c. c. This would have the effect of making still more pronounced the point here brought out.

[2] After deducting the figure for carbon dioxid from the total quantity of gas extracted from the tube and assuming that the residual gas is pure air—that is, approximately one-fifth oxygen.

From the above-mentioned data it will be seen that but a slight oxidation of the fat occurred during a storage interval of more than three months at a temperature of 32° F., even when the sample was kept under conditions decidedly more favorable, in comparison with the preceding one, to permit any pronounced oxidation. It would appear, therefore, that any oxidation of pure butter fat kept in storage at a temperature of 0° F. for a reasonable length of time, if it occurs at all, must be extremely slight. The results of experiments already conducted, however, have shown that a progressive oxidation in whole butter may occur while held in storage at a temperature of 0° F.

The question, therefore, arises whether there occurs an oxidation progressing in some one or more of the nonfatty constitutents of whole butter. In the attempt to clear up this point the following experiments were conducted.

## OXIDATION OF NONFATS

The butter samples used in the two following experiments were made in the experimental creamery at Troy, Pa., from the same lot of cream as was the preceding sample of butter fat stored at a temperature of 0° F. The cream was pasteurized in a continuous pasteurizer at a temperature of 165° F., and was ripened with a pure culture. The acidity of the cream at the time of churning was 0.40 per cent (calculated as lactic acid). The butter in the churn was washed until the wash water was just clear. One half of the butter in the churn was removed and was designated as "normally washed butter." The other half, which remained in the churn, was now given an additional copious washing in four changes of water and designated as "excessively washed butter."

The sample designated in the experiments as "unwashed butter" was prepared from a different lot of cream, which was pasteurized and ripened under the same conditions as indicated above. It was ripened to an acidity of 0.51 per cent (calculated as lactic acid), cooled to 7½° C. (45.5° F.), held overnight, during which the acidity rose to 0.65 per cent, and then churned. The buttermilk was drawn off and the butter allowed to remain unwashed, so as to contain the greatest amount of nonfatty ingredients of all three samples.

Since it was desired to have the three foregoing samples differ from one another only with respect to their buttermilk content, care was taken to prepare them otherwise in identically the same manner. Each was worked on a table worker to the extent of 40 revolutions, to incorporate a large quantity of air. They were then packed in clean and sterile glass jars, and also in the special glass tubes for air analysis. The butter in the jars was covered with a thin layer of paraffin to exclude any action of the atmosphere other than that confined within the material itself.

The appearance of undesirable flavors in stored butter has often been attributed to the use of either impure salt or water, or both, so this contingency was avoided by the use, in all cases, of chemically pure sodium chlorid and distilled water.

The samples were shipped by express to Washington, D. C., where they were kept in cold storage at a temperature of 0° F. Samples taken from the various lots packed in the jars were at once analyzed, and, in addition, were scored by Messrs. Corneliuson and Rabild, of the Dairy Division. After intervals of approximately one month, samples were withdrawn from storage, analyzed, and scored. This was continued for several months, during which time a sufficient period had elapsed for the samples to manifest any change which might occur in butter stored for a reasonable length of time. Of the three samples, designated for convenience as "excessively washed butter," "normally washed butter," and "unwashed butter," the first two will be given and discussed in conjunction (Tables VIII and IX).

TABLE VIII.—*Scores of excessively washed butter, with low content of nonfatty ingredients, stored at 0° F.*

[Protein, 0.50 per cent. Total N×6.38]

| Age. | Score. | Remarks. | Scorer. |
|---|---|---|---|
| *Months.* | | | |
| 1 | 90 | Good, but trifle stale | Corneliuson. |
| 3 | 87 | .....do | Do. |
| 5 | 88 | .....do | Do. |
| 6 | 87 | .....do | Do. |

TABLE IX.—*Scores of normally washed butter, with normal content of nonfatty ingredients, stored at 0° F.*

[Protein, 0.57 per cent. Total N×6.38]

| Age. | Score. | Remarks. | Scorer. |
|---|---|---|---|
| *Months.* | | | |
| 1 | 91 | Flavor good | Corneliuson. |
| 2 | 89 | Trifle stale | Do. |
| 3 | 87 | Aroma good, trifle stale | Rabild. |
| 5 | 88 | Flavor good | Corneliuson. |
| 6 | 88 | .....do | Do. |

The keeping qualities of the two foregoing samples were practically the same, as shown by the scoring. The determination of the chemical constants gave the data in Tables X and XI.

TABLE X.—*Chemical constants of the fat of excessively washed butter, with low content of nonfatty ingredients, stored at 0° F.*

[Protein, 0.50 per cent. Total, N×6.38]

| Age. | Reichert-Meissl number. | Iodin number. | Saponification number. | Soluble acids as butyric. | Insoluble acids. | Acetyl value. | Free acid as oleic. |
|---|---|---|---|---|---|---|---|
| | | | | *Per cent.* | *Per cent.* | | *Per cent.* |
| Initial | 30.03 | 37.30 | 226.8 | 5.552 | 87.54 | 3.703 | 0.456 |
| 2 months | 29.83 | 36.52 | 226.4 | 5.623 | 87.63 | 3.578 | .458 |
| 4 months | 29.89 | 36.42 | 225.9 | 5.130 | 87.58 | 3.535 | .413 |

TABLE XI.—*Chemical constants of the fat of normally washed butter, with medium content of nonfatty ingredients, stored at 0° F.*

[Protein, 0.57 per cent. Total, N×6.38]

| | Reichert-Meissl number. | Iodin number. | Saponification number. | Soluble acids as butyric. | Insoluble acids. | Acetyl value. | Free acid as oleic. |
|---|---|---|---|---|---|---|---|
| | | | | *Per cent.* | *Per cent.* | | *Per cent.* |
| Initial | 30.03 | 37.30 | 226.8 | 5.552 | 87.54 | 3.703 | 0.450 |
| 2 months | 30.64 | 36.39 | 226.5 | 6.072 | 87.03 | 3.581 | .447 |
| 3 months | 30.16 | 36.98 | 226.1 | 5.400 | 87.50 | 3.042 | .459 |
| 5 months | 29.78 | 36.52 | 226.6 | 5.150 | 87.44 | 3.323 | .412 |

There is practically no variation in these figures from those obtained in the foregoing determination of chemical constants with the nearly pure butter fat standard. Evidently the fat in these two samples of butter underwent little or no chemical change, owing to the presence of either the confined air or the other nonfatty components. The analysis of this confined air, however, gave figures which differed considerably from those obtained in the analysis of the air confined within the samples of the butter fat itself (Table XII).

TABLE XII.—*Analysis of air in excessively washed butter, with low content of nonfatty ingredients, stored at 0° F.*

[Calculated to 0° C. and 760 mm. Protein, 0.50 per cent. Total, N×6.38]

| Age. | Total gas. | Total carbon dioxid. | | Total oxygen. | | Calculated oxygen.[1] |
|---|---|---|---|---|---|---|
| *Months.* | *C. c.* | *C. c.* | *Per cent.* | *C. c.* | *Per cent.* | *C. c.* |
| 2 | 31.88 | 4.48 | 14.05 | 5.52 | 17.31 | 5.50 |
| 3 | 42.50 | 2.77 | 6.52 | 6.47 | 15.23 | 7.95 |
| 4 | 26.57 | 2.01 | 7.57 | 3.39 | 12.70 | 4.91 |
| 5 | 26.28 | 1.94 | 7.38 | 3.24 | 12.33 | 4.87 |
| 14 | 30.55 | 1.74 | 5.70 | 1.84 | 6.03 | 5.78 |

[1] After deducting figure for carbon dioxid from total quantity of gas extracted from the tube and assuming that the residual gas is pure air—that is, approximately one-fifth oxygen.

TABLE XIII.—*Analysis of air in normally washed butter, with medium content of nonfatty ingredients, stored at 0° F.*

[Calculated to 0° C. and 760 mm. Protein, 0.57 per cent. Total, N×6.38]

| Age. | Total gas. | Total carbon dioxid. | | Total oxygen. | | Calculated oxygen.[1] |
|---|---|---|---|---|---|---|
| *Months.* | *C. c.* | *C. c.* | *Per cent.* | *C. c.* | *Per cent.* | *C. c.* |
| 2 | 27.88 | 1.85 | 6.64 | 5.26 | 18.87 | 5.21 |
| 3 | 29.99 | 3.07 | 13.24 | 4.34 | 14.47 | 5.20 |
| 4 | 26.26 | 3.45 | 13.14 | 3.72 | 14.17 | 4.56 |
| 14 | 28.94 | 3.90 | 13.47 | 2.41 | 8.33 | 5.01 |

[1] After deducting figure for carbon dioxid from total quantity of gas extracted from the tube and assuming that the residual gas is pure air—that is, approximately one-fifth oxygen.

There is no great difference in the total quantity of carbon dioxid to be observed between these samples of excessively washed butter and what is considered to represent normally washed butter. It is to be noted that there is very little difference in the protein content of these two

samples. The high point (14.05 per cent) for carbon dioxid reached in the first case is about the same as that reached in the second (13.47 per cent), while there is no wide variation in the oxygen content of the two samples. The great decrease in the percentage of carbon dioxid in the former case occurs in the interval between the second and third months, after which this decreased percentage remains fairly constant. This decrease in the original percentage of carbon dioxid is also accompanied with a pronounced decrease in the percentage of oxygen. In the latter case the percentage of carbon dioxid increases to its maximum after the sample has been three months in storage, after which it remains fairly constant. It is to be noted, however, that the total amount of oxygen originally present in these samples of butter containing a certain proportion of buttermilk undergoes a markedly progressive decrease during the interval that the butter is kept in storage at a temperature of 0° F.

A survey of the data obtained from the sample of unwashed butter is of additional interest in this connection (Table XIV).

TABLE XIV.—*Scores of unwashed butter, with high content of nonfatty ingredients stored at 0° F.*

[Protein 0.90 per cent. Total N×6.38]

| Age. | Score. | Remarks. | Scorer. |
|---|---|---|---|
| *Months.* | | | |
| 1 | 92 | Oily, mottled | Corneliuson. |
| 2 | 89 | Oily, unclean | Rabild. |
| 3 | 87 | ....do | Corneliuson. |
| 5 | 85 | Slightly fishy | Do. |
| 6 | 86 | Stale, fishy, sour | Do. |

The progressive development of "off flavor" in this sample of butter, so prepared as to contain a greater quantity of buttermilk than either of the two foregoing samples, was remarkable. Since this butter had been prepared from a different lot of cream, it was necessary to establish a new fat standard of constants. The butter fat for this purpose was prepared from the same lot of cream as was the butter, and it was packed and stored in the same manner as that given for the previously mentioned sample of butter fat. The results are given in Tables XV and XVI.

TABLE XV.—*Chemical constants of butter fat stored at 0° F after being nearly freed from the nonfatty ingredients by melting, filtering, and washing*

| Age. | Reichert-Meissl number. | Iodin number. | Saponification number. | Soluble acids as butyric. | Insoluble acids. | Acetyl value. | Free acid as oleic. |
|---|---|---|---|---|---|---|---|
| | | | | *Per cent.* | *Per cent.* | | *Per cent.* |
| Initial | 26.16 | 41.91 | 226.3 | 5.275 | 86.93 | 3.365 | 0.210 |
| 2 months | 26.90 | 41.40 | 225.9 | 5.147 | 87.46 | 3.579 | .220 |
| 3 months | 26.71 | 40.76 | 226.2 | 5.263 | 87.65 | 3.260 | .222 |
| 5 months | 26.93 | 40.79 | 226.5 | 5.220 | 87.55 | 3.335 | .214 |
| 6 months | 26.84 | 40.88 | 225.1 | 5.166 | 87.38 | 3.397 | .225 |

TABLE XVI.—*Chemical constants of the fat of unwashed butter, with high content of nonfatty ingredients, stored at 0° F.*

[Protein 0.90 per cent. Total N×6.38]

| Age. | Reichert-Meissl number. | Iodin number. | Saponification number. | Soluble acids as butyric. | Insoluble acids. | Acetyl value. | Free acid as oleic. |
|---|---|---|---|---|---|---|---|
| *Months.* | | | | *Per cent.* | *Per cent.* | | *Per cent.* |
| 1 | 26.28 | 41.80 | 226.6 | 5.29 | 87.1 | 3.90 | 0.205 |
| 2 | 26.76 | 40.40 | 226.4 | 5.06 | 86.66 | 3.795 | .210 |
| 3 | 26.90 | 40.81 | 226.3 | 5.32 | 87.02 | 3.381 | .226 |
| 5 | 26.83 | 40.13 | 226.5 | 5.27 | 87.44 | 3.331 | .231 |
| 6 | 26.84 | 40.30 | 225.0 | 5.17 | 86.98 | 3.294 | .226 |

The same comments are here to be made as in the case of the previous sample of nearly pure butter fat. No noteworthy chemical change had occurred in this sample of butter fat after having been kept in storage at a temperature of 0° F. for a period of six months. With respect to the fat taken from the sample of butter so prepared as to contain the greatest number of constituents in addition to the fat, the same observations are here to be made as in the previous cases of two different lots of butter containing smaller numbers of nonfatty constituents. The chemical constants here show little or no variation from those obtained with the nearly pure butter fat, and there is apparently no chemical change in the fat of butter prepared with a still greater number of substances in addition to the fat, owing either to the presence of these substances or to the presence of the confined air. An analysis of this confined air, however, gives some very striking data (Table XVII) when compared with those obtained in the foregoing samples.

TABLE XVII.—*Analysis of air in unwashed butter, with high content of nonfatty ingredients, stored at 0° F.*

[Calculated to 0° C. and 760 mm. Protein 0.90 per cent. Total N×6.38]

| Age. | Total gas. | Total carbon dioxid. | | Total oxygen. | | Calculated oxygen.[1] |
|---|---|---|---|---|---|---|
| *Months.* | *C. c.* | *C. c.* | *Per cent.* | *C. c.* | *Per cent.* | *C. c.* |
| 2 | 29.26 | 9.19 | 31.41 | 0.71 | 2.43 | 4.01 |
| 3 | 28.23 | 8.94 | 31.67 | .62 | 2.20 | 3.86 |
| 4 | 29.15 | 8.13 | 27.89 | .47 | 1.61 | 4.20 |
| 5 | 38.19 | 9.97 | 26.12 | .37 | .97 | 5.64 |
| 15 | 33.28 | 7.41 | 22.26 | .37 | 1.11 | 5.17 |

[1] After deducting the figure for carbon dioxid from the total quantity of gas extracted from the tube and assuming that the residual gas is pure air—that is, approximately one-fifth oxygen.

The maximum content of carbon dioxid (31.67 per cent) in this sample of unwashed butter was noticed after a storage period of three months, at about which time the characteristic "off flavor" became distinctly noticeable. At the end of two months there was very little oxygen in the sample; yet even this shows a perceptible decrease during the storage interval.

It has been indicated in the foregoing experiments that the quantity of carbon dioxid occurring in the gas inclosed in a package of stored butter is proportional to the amount of nonfatty ingredients incorporated into the material. There is also a more or less pronounced decrease in the oxygen content during the storage period. The following additional experiment was made with the view of confirming this relative change in the percentages of carbon dioxid and oxygen, and especially to determine the quantities of these gases occurring in unwashed butter at the time of its manufacture, since all the above-described analyses were made upon the samples after an interval of two months in storage.

For this purpose some unwashed butter was prepared from cream pasteurized at 145° F. for 20 minutes, and, as in the other cases, ripened with a pure culture. The cream was ripened to an acidity of 0.45 per cent (calculated as lactic acid), cooled to 7° C. (44.6° F.), held overnight, during which the acidity rose to 0.67 per cent, and then churned. The buttermilk was drawn off and the butter allowed to remain unwashed. The butter was then salted with chemically pure salt and worked on a table worker. This butter contained 4.72 per cent of sodium chlorid and 0.56 per cent of protein (total N×6.38). The butter was then packed into the special glass tubes for air analysis.

The gas in the first sample was extracted therefrom and analyzed as soon as possible after the butter was made—that is, 1½ hours. The remaining samples were kept at room temperature, but in the dark (Table XVIII).

TABLE XVIII.—*Analysis of air from a second sample of unwashed butter kept at room temperature but in the dark*

[Calculated to 0° C. and 760 mm. Protein 0.56 per cent. Total N×6.38]

| Age. | Total gas. | Total carbon dioxid. | | Total oxygen. | | Calculated oxygen.[1] |
|---|---|---|---|---|---|---|
| | *C. c.* | *C. c.* | *Per cent.* | *C. c.* | *Per cent.* | *C. c.* |
| 1½ hours | 37.7 | 7.5 | 19.89 | 7.7 | 20.42 | 6.0 |
| 2 days | 33.4 | 7.4 | 22.16 | 5.6 | 16.77 | 5.2 |
| 7 days | 33.8 | 7.5 | 22.19 | 5.1 | 15.09 | 5.3 |
| 14 days | 35.8 | 8.2 | 22.91 | 3.8 | 10.61 | 5.5 |

[1] After deducting the figure for carbon dioxid from the total quantity of gas extracted from the tube and assuming that the residual gas is pure air—that is, approximately one-fifth oxygen.

Most of the carbon dioxid appears to have existed in the butter as soon as the manufacture of the material was completed. It also appears to increase somewhat in quantity during a period of two weeks. The oxygen figures show in a striking manner the decrease in the initial quantity of this gas present in the butter, and it is apparent that it has decreased to practically one-half this quantity after being kept two weeks at room temperature in the dark.

The question now arises whether there exists in the samples of butter fat the same homogeneous distribution of air bubbles as in the case of those samples of butter containing the varying quantities of nonfatty ingredients, for it is conceivable that the air incorporated into the butter fat may occur mostly in large pockets, while the other samples may contain, in addition, a certain amount of the total air inclosed within the particles of curd, lactose, etc.

In the first case it is reasonable to suppose that a smaller surface of material would be exposed to the influence of the air than in the second; yet it is improbable that this would alter the basic facts, since the analytical data obtained in the experiments indicate that the particles of nonfatty ingredients inclosing the air are more readily attacked by the oxygen therein than the fat itself. However, to obtain further confirmatory data on this point—that is, that the nonfatty constituents of butter are more readily attacked by the oxygen of the air incorporated into the material than the fat itself—the following experiments were conducted.

## BUTTERMILK EXPOSED TO A LARGE SURFACE OF AIR

Several of the special butter tubes were filled with large fragments of cracked and ignited pumice. The pumice of one lot of tubes was impregnated with the buttermilk from butter made from pasteurized cream acidified to 1 per cent with lactic acid before churning. The pumice of each tube of a second lot was treated with 10 c. c. of a 1 per cent solution of lactic acid. The tubes of these two lots were kept at a temperature of 32° F. At various times tubes from each were removed from storage and an analysis of the air in them was made. The analytical data obtained are given in Table XIX.

TABLE XIX.—*Oxidation of acid-cream buttermilk and of lactic acid exposed to the action of a large surface of air at a temperature of 32° F.*

| Acid buttermilk. | | | Lactic acid. | | |
|---|---|---|---|---|---|
| Period at 32° F. | Oxygen. | Carbon dioxid. | Period at 32° F. | Oxygen. | Carbon dioxid. |
| *Days.* | *Per cent.* | *Per cent.* | *Days.* | *Per cent.* | *Per cent.* |
| 4½ | 17.67 | 2.37 | 30 | 20.70 | 0 |
| 26 | 0 | 34.37 | 60 | 21.07 | 0 |
| 62 | 0 | 31.76 | 98 | 21.01 | 0 |

The change in the composition of the air in contact with the acid buttermilk was very marked during a storage interval of only 26 days when this sample was kept at a temperature of 32° F. From a total percentage of 17.67 found to be present in the acid buttermilk when the material was 4½ days old, the oxygen content fell to zero during the period between this time and 26 days. The carbon-dioxid content of the buttermilk, initially small in quantity, rapidly increases to a maxi-

mum, from which point it begins to decrease. It is also very clear from the control experiment given in Table XIX that the change in the composition of the air inclosed in the material is not caused by decomposition of the lactic acid itself or to any action of this acid upon the particles of pumice.

It has already been shown in this paper that the acidity of the cream from which the butter is made has a direct influence on the change, during storage at a temperature of 0° F., in the composition of the air incorporated into the butter at the time of its manufacture. It has also been shown that but a slight change is to be observed in the composition of the air from a tube containing a small quantity of pure butter fat exposed to the action of a large and confined surface of air while the fat was kept at a temperature of 32° F. It has likewise been demonstrated that practically no change in the composition of the air occurs when pure butter fat is exposed to the action of about the same amount of air as is usually present in normal butter while it is stored at a temperature of 0° F. As it has been proved by an analysis of the air from a sample of sweet-cream butter made from cream of low acidity that this kind of butter suffers very little, if any, measurable decomposition during a period of six months in storage at a temperature of 0° F., and having also proved by other experiments that a decomposition of the fat of whole butter stored at a temperature of 0° F. for the same length of time is practically excluded, it is a logical conclusion that the particles of buttermilk inclosed in a sample of sweet-cream butter made from cream of low acidity likewise suffers little, if any, measurable decomposition when the butter is stored at a temperature of 0° F. It was decided, however, to settle this point definitely by experiment.

For this purpose the pumice of a third lot of tubes was impregnated with buttermilk from sweet-cream butter made from pasteurized cream having an acidity of 0.108 per cent (calculated as lactic acid). The acidity of the cream in this case was practically the same as that of the cream from which the foregoing sample of sweet-cream butter was made. This last lot of tubes containing the sweet-cream buttermilk was kept at a temperature of 0° F. (Table XX).

TABLE XX.—*Oxidation of sweet-cream buttermilk exposed to the action of a large surface of air at 0° F.*

| Period at 0° F. | Oxygen. | Carbon dioxid. |
|---|---|---|
| *Days.* | *Per cent.* | *Per cent.* |
| 35 | 20.92 | 0 |
| 65 | 20.93 | 0 |
| 270 | 20.25 | 0 |

That the sweet-cream buttermilk underwent practically no change in storage at a temperature of 0° F. is shown by the foregoing data.

## SUMMARY AND CONCLUSIONS

The composition of the air confined within a package of pasteurized sweet-cream butter known to contain bacteria and made from cream having an acidity of 0.11 per cent (calculated as lactic acid) showed little or no variation from its original composition after successive periods in storage, aggregating six months, at a temperature of 0° F. A small quantity of the buttermilk from butter made from pasteurized sweet cream having the same low degree of acidity as the cream above mentioned, when exposed to the influence of a very large and confined surface of air, appeared to have little, if any, effect upon the original composition of the air when the buttermilk was stored for nine months under like conditions of temperature. A portion of this same sample of sweet-cream butter when kept at a temperature of 32° F. showed a decided change in the original composition of the inclosed air, a change which was still further increased when the butter remained for a short time at room temperature. This change in the composition of the air originally incorporated into the butter was expressed by a decrease in the percentage of oxygen and a corresponding increase in the percentage of carbon dioxid. This sample of sweet-cream butter still possessed a good score after six months' storage at a temperature of 0° F., there being no indication of any undesirable flavor.

The change in the composition of the air initially inclosed within a package of butter made from sweet cream and churned immediately after the addition of 15 per cent of a commercial starter showed but little variation from that observed in the sample of sweet-cream butter when the two samples were kept under comparable conditions, both being in storage at a temperature of 0° F., although the acidity of the cream in the first case was somewhat higher (0.25 per cent) than that of the cream from which the sweet-cream butter was made. This sample of butter also displayed good keeping qualities during its storage period of nearly seven months at a temperature of 0° F.

The composition of the air inclosed within a package of butter made from sweet cream and churned immediately after the addition of lactic acid, the total acidity of the cream being about six and one-half times greater than that of the cream from which the sweet-cream butter was made, showed pronounced variations from its original composition during successive periods of storage at a temperature of 0° F. These variations were still greater when the sample was allowed to stand at a temperature of 32° F. In this case there was a considerable and a progressive decrease in the original oxygen content, as well as in the original carbon-dioxid content. A small quantity of the buttermilk from butter made from pasteurized sweet cream and churned immediately after the addition of lactic acid, when exposed to the action of a very large and confined surface of air under the same temperature conditions, showed precisely the

same phenomena with respect to alteration in the original air composition. The oxygen content of the confined air had entirely disappeared within a month's time. The carbon-dioxid content, originally 2.37 per cent, had increased to more than 34 per cent within the same interval, after which time it had begun to decrease. The flavor of this butter, which was prepared from pasteurized sweet cream and churned immediately after the addition of lactic acid, was somewhat unclean after a storage period of only three months at a temperature of 0° F., and decidedly so after being in storage for six months under the same conditions.

Further, it has been indicated by the investigation pursued with pasteurized, ripened-cream butter through the successive steps from nearly pure butter fat to samples of butter containing varying quantities of ingredients other than fat and, finally, to samples containing the greatest quantity of protein, lactose, etc., and stored for a reasonable length of time (six months) at a temperature of 0° F., that the amount of carbon dioxid inclosed in a package of the material is directly proportional to the quantity of these ingredients contained therein. It has been shown that this quantity of carbon dioxid may increase during the earlier part of the storage period, followed by a decrease during the latter part. It is also of especial significance, perhaps, that the oxygen content of the gas in the material undergoes a marked and striking decrease during the interval that the samples of butter containing the varying amounts of constituents other than fat are retained in storage, and that this decrease is likewise proportional to the amount of acid and ingredients other than fat contained in the butter.

The fat of butter made from pasteurized cream, on the contrary, undergoes no apparent oxidation during the same storage period when kept at a temperature of 0° F. It is only when a substance like pumice, which may have catalytic properties, is impregnated with a small amount of butter fat and exposed to the action of a large amount of air while kept at a temperature of 32° F. that a very slight oxidation is noticeable.

The results of the investigations may be summed up as follows:

(1) The development of undesirable flavors in butter held in cold storage at a temperature of 0° F. is not dependent upon an oxidation of the fat itself.

(2) The production of "off flavors" so commonly met with in cold-storage butter is attributable to a chemical change expressed through a slow oxidation progressing in some one or more of the nonfatty substances occurring in the buttermilk.

(3) The extent of this chemical change is directly proportional to the quantity of acid present in the cream from which the butter was prepared.

(4) The quantity of carbon dioxid present in cold-storage butter appears to have a certain relation to the quantity of buttermilk in the butter. During storage this quantity of carbon dioxid may increase to a maximum followed by a progressive decrease.

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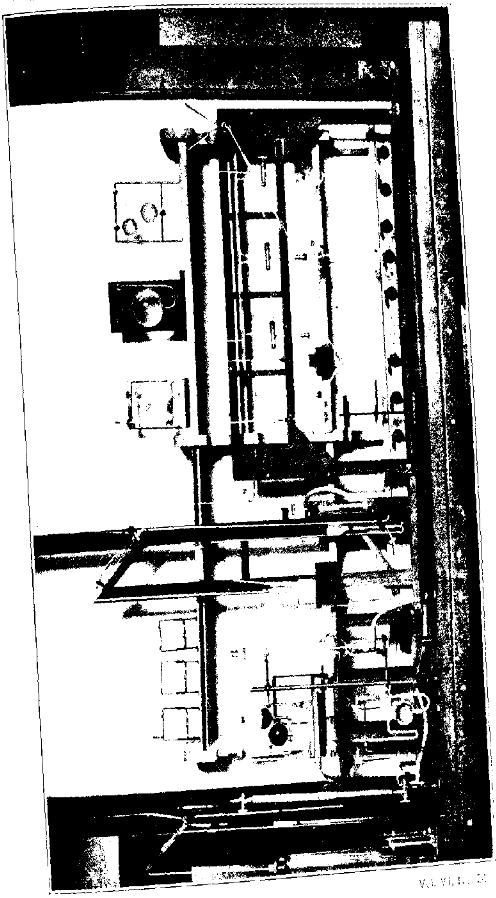
PLATE CXI

Gas apparatus used in the extraction and analysis of the air confined in butter.



# BACTERIOLOGICAL STUDIES OF A SOIL SUBJECTED TO DIFFERENT SYSTEMS OF CROPPING FOR TWENTY-FIVE YEARS

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## INTRODUCTION

During the past few years a number of soil biologists have reported their findings regarding the effects of different agricultural practices upon the bacteria of the soil. The majority of these investigations have been concerned with determining the gross effects of a particular treatment upon the physiological activities of the flora as a whole. Such factors as continuous cropping, rotational systems, cultural methods, application of chemical fertilizers, and manures have been studied. Among the more recent workers reporting such investigations in the United States may be mentioned King and Doryland (10),[1] Stevens and Withers (18), Stewart and Greaves (19), Lyon and Bizzell (12), Temple (20), Jensen (9), Given and Willis (6, 7), Brown (2, 3, 4), Hill (8), Allen and Bonazzi (1), Wright (22), and McBeth and Smith (13).

It is not necessary to give any extensive review of the work that has been done as the papers referred to above contain full summaries of the results thus far obtained. Special attention may be called to the review given by Temple (20) as to the effect of stable manure, by Hill (8) as to the effect of other organic materials, and by Lyon and Bizzell (12) as to the effect of different growing crops. The available data leave little doubt that certain of the above-mentioned factors do exert a marked effect upon soil organisms. In very few instances, however, has any serious effort been made to ascertain just how such factors exert their influence. In most instances the treatment in question has been in operation a comparatively short time. Brown (3), for example, studied the effects of a 4-year rotation while the fourth crop was still on the soil, or before the cycle was completed the first time. It is true that the quantities of nitrate nitrogen have been determined *in situ*, following long-continued cropping systems. With our present very limited knowledge as to the demands any particular crop makes upon soil nitrates, such information gives us little insight into nitrate formation. Allen and Bonazzi (1) carried out a few laboratory experiments, following a long-continued treatment, but obtained such irregular results that they regarded them of little value. Given and Willis (6, 7) have also re-

[1] Reference is made by number to "Literature cited," p. 974-975.

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ported a limited number of experiments following 30 years of continuous treatment.

It was with the ultimate object of attempting a study of the fundamental causes for variations in certain changes in soil nitrogen that this work was undertaken. Before it could be begun, however, it was first necessary to determine the existence of differences under our conditions. Furthermore, it seemed desirable to compare the relative effect of long-continued treatment with that of the shorter periods reported by others.

The Missouri Experiment Station possesses a series of fertility plots that offered exceptional opportunity for making the above-mentioned studies. The plots cover a rather wide range and have just passed their twenty-fifth year of continuous treatment as outlined in the original project. Some very valuable data as to the effect upon fertility as measured by crop-producing power have been obtained. It would seem as if 25 years would have so materially changed the micro-organic life therein that such could readily be detected, provided such differences were actually brought about. With such material upon which to work it would seem that the verity of marked differences in similarly treated soil reported by others could be established.

The data herein reported have been obtained from some of the plots that have given the most marked differences in yield, as it was believed such plots would offer the best material upon which to work. The work, however, is concerned only with demonstrating the existence of differences and offers only one or two suggestions as to the actual cause of such differences. We hope to be able to throw more light upon this particular field at a later date. Furthermore, the particular data here reported have to do only with bacterial numbers and with ammonia and nitrate-forming abilities.

Certain facts, which, we believe, we have very clearly demonstrated, have been of value to us in directing further work. It is with the hope that the data may be of similar value to others that we present them in this paper.

## PLOTS STUDIED

The fertility plots of the Missouri Experiment Station are located on the soil type classed as Putnam silt loam. They were first planted to the present system in 1889 and, with few irregularities, have received the same treatment as outlined. Each plot consists of one-tenth acre and is surrounded by an alley 3 feet wide. In selecting from the large number of plots the few that could be handled in our work, an effort was made to include as wide a variation of treatment as possible and at the same time to avoid inherent soil differences in order that the work might be comparative.

The plots studied are, with the exception of No. 29 and 30, located just at the crest and on the eastern slope of a gentle rise. Plots 29 and

30 are of a slightly different texture, being located on the western slope of the same rise. For this reason they are omitted from the general scheme and are compared with each other only. The greatest distance between any two plots (No. 1 and 23) is only about 85 yards. It was impossible to get the specially treated plots that we wished to study located any closer together. It is not believed, however, that any difference due to character of soil, location, etc., could materially affect the major differences reported. The plots studied, treatment received, and yields are given in Table I. It will be noted that the plots studied during 1914 varied somewhat from those of the previous year. This was necessary because of a change in treatment in certain plots beginning in 1914. In the tabulated data, plot 1 always includes the data obtained from plot 20 (a duplicate). Similarly plot 10 includes data secured from plot 21 (a duplicate).

Where stable manure has been applied, it has been an annual application averaging 6.7 tons per acre on all plots except No. 1, which received 7 tons. The chemicals were applied annually on plots 2 and 3 in the form of sodium nitrate, potassium chlorid, and acid phosphate in quantities sufficient to supply nitrogen, potassium, and phosphorus for a full yield of the particular crop. In the case of wheat this was for a yield of 40 bushels. The rotation consisted of corn, oats, wheat, clover, timothy, and timothy. The other plots have been annually planted to the specific crop mentioned.

TABLE I.—*Cropping system, treatment, and yields of the various fertility plots studied at Columbia, Mo.*

| Plot No. | Cropping system. | Treatment. | Year studied. | Yield. | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 1914 | 1913 | 1909–1913 | 1904–1913 | 1889–1913 |
| 1 | 6-year rotation [a] | Stable manure | 1914 | 1,326 pounds | 3,887 pounds | | | |
| 2 | Continuous wheat | Chemicals | 1914 | 20.32 bushels | 17.01 bushels | 12.83 bushels | 16.38 bushels | 17.99 bushels. |
| 3 | 6-year rotation | ...do | 1914 | 964 pounds | 2,664 pounds | | | |
| 9 | Continuous wheat | None | 1914 | 20.69 bushels | 8.83 bushels | 6.41 bushels | 6.54 bushels | 9.56 bushels. |
| 10 | ...do | Stable manure | 1914 | 21.37 bushels | 16.90 bushels | 12.21 bushels | 12.47 bushels | 16.72 bushels. |
| 13 | 6-year rotation | None | 1913–14 | 377 pounds | 1,508 pounds | | | |
| 17 | Continuous corn | ...do | 1913–14 | 28.78 bushels | 6.96 bushels | 10.08 bushels | 10.68 bushels | 20.35 bushels. |
| 18 | ...do | Stable manure | 1913–14 | 33.89 bushels | 19.22 bushels | 19.64 bushels | 24.54 bushels | 35.78 bushels. |
| 20 | 6-year rotation | ...do | 1913 | 1,177 pounds | 4,810 pounds | | | |
| 21 | Continuous wheat | ...do | 1913 | 28.31 bushels | 17.44 bushels | 14.35 bushels | 15.82 bushels | 18.66 bushels. |
| 22 | Continuous timothy | ...do | 1913–14 | 1,482 pounds | 3,042 pounds | 4,388 pounds | 4,345 pounds | 5,006 pounds. |
| 23 | ...do | None | 1913–14 | 910 pounds | 1,339 pounds | 2,075 pounds | 2,264 pounds | 2,853 pounds. |
| 29 | Continuous wheat | Stable manure [b] | 1913 | 29.56 bushels | 16.79 bushels | 11.87 bushels | 12.59 bushels | 15.6[illegible] bushels. |
| 30 | ...do | ...do | 1913 | 28.17 bushels | 22.53 bushels | 12.66 bushels | 14.96 bushels | 19.58 bushels. |

[a] The 6-year rotation plots were in clover during 1913 and timothy during 1914.

[b] Stable manure since 1908.

## EXPERIMENTAL METHODS EMPLOYED

Practically all the methods here mentioned have been severely criticised during the past few years, particularly by Löhnis and Green (11), Allen and Bonazzi (1), and Noyes (14). However, in our laboratory the methods described below have proved, for the object in view, equal or superior to any suggested prior to the beginning of this work.

Samples for the various analyses were taken with a 1½-inch soil auger. Ten to fifteen samples were collected from each plot to the depth of the soil, which was about 10 inches. The cores of soil were taken uniformly all over the plot, avoiding close proximity to the surrounding alleys. They were placed immediately in sterile Mason jars in order to prevent loss of moisture and to avoid contamination, so far as possible. The samples were then brought to the laboratory as soon as possible, where, under aseptic conditions, the soil was passed through a 2-mm. sieve and thoroughly mixed. Samples were immediately taken for quantitative analyses and for moisture determinations.

For moisture determinations 50 gm. of soil were dried at 110° C. for two hours. To determine the water-holding capacity, 50 gm. were placed in a carbon filter containing a perforated porcelain bottom and a measured quantity of water poured on top. The water was permitted to percolate through the soil. The process was repeated two or three times. From the amount of water absorbed plus the quantity lost in drying, the water-holding capacity, expressed in grams of water held per 100 gm. of dry soil, was determined. Data obtained by these methods are, of course, not absolute. But the process possesses two essentials: Quickness of manipulation and comparativeness. Since slight differences in water content, when near the optimum, exercise but little influence upon bacterial activity, it is believed the error introduced is not appreciable.

Quantitative analyses were made by carefully weighing 1 or 2 gm. of soil, placing it in 98 or 99 c. c. of sterile water and shaking vigorously for one minute. From this suspension dilutions were made in the ordinary way. Finally, 1 c. c. was placed in each of three sterile Petri dishes and thoroughly mixed with 10 c. c. of Temple's agar (20). The dishes were then incubated for one week at room temperature and all colonies counted with the aid of a hand lens. If one of the dishes varied widely from the other two it was discarded. The same was true if dishes were overrun by spreaders or molds. The results are reported in millions of bacteria per gram of dry soil.

The ammonia- and nitrate-forming experiments were carried out by thoroughly mixing into fresh soil (the equivalent of 100 gm. of dry soil) sterile cottonseed meal containing 60 mgm. of nitrogen. This was placed in a sterile 500 c. c. wide-mouthed bottle and the moisture con-

tent made up to the optimum (two-thirds water-holding capacity). Two samples were incubated for one week and two for four weeks. The ammonia and nitrate nitrogen were then determined and reported as milligrams of nitrogen and nitrates ($NO_3$), respectively, per 100 gm. of soil. In 1913 the ammonia was determined as follows: The water content was made up to a definite volume and the whole shaken for 45 minutes. Two gm. of calcium oxid were then added and the contents were again shaken for a short time and allowed to stand until the supernatant liquid became clear. A definite volume of this liquid was then distilled in the presence of magnesium oxid, the distillate being collected in standard acid and titrated. The calcium oxid was added as a clarifying agent in order to obtain a solution upon which nitrate and nitrite nitrogen could be determined colorimetrically. The presence of this reagent caused a perceptible increase in the ammonia set free, probably liberating some of the loosely attached nitrogen; but since the results are comparative and because of reasons already mentioned, the method seemed justifiable. However, such insignificant quantities of nitrate and nitrite nitrogen were found after seven days' incubation that these determinations were discontinued during 1914, and the ammonia was determined by direct distillation of the soil in copper flasks.

Where the incubation lasted for four weeks, the water loss by evaporation from the soil was replaced from time to time. Besides these experiments, samples were also run during 1914 with the addition of calcium carbonate in excess of that required to neutralize all nitric acid that could be formed from the cottonseed meal. Nitrate nitrogen was determined in all cases upon an aliquot part of a solution, obtained as directed above, using the phenoldisulphonic-acid colorimetric method.

The nitrifying inoculation experiments were conducted as follows: A soil possessing both a high nitrifying capacity and nitrifying efficiency, in the sense that Stevens and Withers (17) use these terms, was selected as a standard medium. To 100-gm. samples of this soil, cottonseed meal containing 60 mgm. of nitrogen was added, sufficient water added to bring it up to optimum, less 20 c. c., and the whole subjected to 20 pounds' pressure for one hour in the autoclave. These samples were then inoculated from the various plots with 20 c. c. of a soil suspension made by shaking 1 part of soil in 2 parts of water. The incubation covered a period of 28 days at room temperature. Ammonia and nitrates were determined as stated above. The results are reported in milligrams of nitrogen and nitrate ($NO_3$), respectively, per 100 gm. of soil. It will be noted that the nitrate data for 1913 are low, in many places zero. This was caused by the failure to add calcium carbonate which was added in 1914. Apparently some substance toxic to nitrification is produced by heating. This substance gradually disappears on standing, and the disappearance is materially hastened by the addition of calcium carbonate.

The cross-inoculation experiments were tested by taking a mixture of all samples collected during both seasons from the respective plots, thoroughly mixing, and using as a medium 100-gm. samples containing 60 mgm. of nitrogen. These samples were also subjected to 20 pounds' pressure for one hour in the autoclave. A sufficient number of samples were thus prepared for duplicate inoculation from each plot under study. One of the duplicates received calcium carbonate; the other did not. These samples were incubated for six instead of four weeks and nitrate nitrogen determined as before.

In all the above-outlined experiments duplicate samples were set up and analyzed. Where possible, as in the nitrate determinations, duplicate determinations were run with each sample. If these varied widely, they were again run; or where this was impossible, they were discarded. In general, the duplicates agreed very well, except in the nitrifying inoculating experiments when incubated for only four weeks with no calcium carbonate added. Perhaps these results should not be included in the tabulated data; but since the relative positions of the averages do not materially differ from those of 1914, they have been included.

## EXPERIMENTAL WORK

### NUMBER OF BACTERIA

Table II gives the moisture content of soil from the different plots at the various samplings.

TABLE II.—*Percentage of moisture in soil of the fertility plots when sampled* [a]

| Plot No. | 1913 | | | | | | 1914 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | July 11. | Aug. 12. | Sept. 12. | Nov. 4. | Dec. 20. | Average. | May 23. | June 22. | July 25. | Sept. 15. | Oct. 30. | Average. |
| 1 | 15.5 | 5.0 | 6.0 | 20.0 | 21.3 | 13.56 | 7.6 | 7.6 | 10.6 | 20.8 | 19.5 | 13.20 |
| 13 | 16.0 | 4.0 | 4.8 | 18.0 | 20.9 | 12.75 | 8.0 | 6.5 | 7.6 | 21.0 | 20.6 | 12.74 |
| 3 | 18.0 | 14.2 | 12.0 | 17.5 | 24.3 | 17.20 | 7.0 | 6.0 | 9.0 | 20.8 | 20.0 | 12.56 |
| 10 | | | | | | | 10.0 | 9.0 | 17.0 | 22.2 | 20.0 | 15.64 |
| 9 | | | | | | | 8.0 | 7.4 | 12.0 | 21.6 | 18.8 | 13.56 |
| 2 | | | | | | | 8.0 | 8.1 | 12.0 | 20.0 | 17.8 | 13.20 |
| 18 | 15.0 | 7.6 | 7.0 | 19.5 | 20.4 | 13.0 | 14.0 | 14.7 | 10.0 | 21.0 | 20.0 | 15.94 |
| 17 | 16.0 | 6.8 | 7.4 | 18.4 | 21.0 | 13.92 | 13.0 | 14.7 | 8.6 | 17.1 | 18.0 | 14.28 |
| 22 | 16.0 | 5.8 | 6.2 | 17.4 | 21.2 | 13.32 | 8.4 | 9.4 | 13.0 | 22.2 | 22.8 | 15.56 |
| 23 | 16.0 | 6.2 | 7.0 | 19.0 | 21.5 | 13.90 | 11.2 | 7.4 | 14.8 | 20.4 | 21.6 | 15.08 |

[a] The soil was dried at 110° C. for two hours.

Table III gives the total number of bacterial colonies developing on Temple's agar expressed in millions per gram of dry soil. More emphasis should be placed upon the 1914 series than the 1913, because of the larger number of analyses and the greater uniformity of plots. A study of this table brings out several interesting facts, the most evident being that of the effect of manure. In all cases except the rotated plot for 1913, all those receiving manure rank materially higher than those not receiving it. Since this exception did not hold true for 1914, it is

possible that some other factor was influencing plot 20. The plots receiving chemical fertilizers ranked a little lower than the lowest receiving manure but materially higher than those receiving nothing.

TABLE III.—*Number of bacteria per gram of soil* [a]

[,000 omitted]

| Plot No. | 1913 | | | | | 1914 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | July 11. | Aug. 12. | Nov. 4. | Dec. 20. | Average. | May 23. | June 22. | July 25. | Sept. 15. | Oct. 30. | Average. |
| 1 | 7.800 | 4.000 | ........ | 1.270 | 4.360 | 5.000 | 4,500 | 4.800 | 8,100 | 7,000 | 5,880 |
| 13 | 3.690 | 7.080 | 4.590 | 1.520 | 4.210 | 2.820 | 2.300 | 4,400 | 4.000 | 5,560 | 3,820 |
| 3 | ........ | ........ | ........ | ........ | ........ | 4.800 | 3.000 | 5,000 | 6.000 | 7,560 | 5,270 |
| 10 | 10.730 | 9.900 | ........ | 6.270 | 8.960 | 7,050 | 4,830 | 11,800 | 15.600 | 26,000 | 13.050 |
| 9 | ........ | ........ | ........ | ........ | ........ | 1.840 | 1,800 | 3,500 | 4.300 | 3,450 | 2,980 |
| 2 | ........ | ........ | ........ | ........ | ........ | 4.730 | 3,150 | 6,480 | 4.560 | 8,100 | 5,400 |
| 18 | 4.600 | 8.000 | 14.000 | 4.820 | 7.855 | 10.400 | 15,400 | 9,000 | 14,750 | 17,100 | 13.330 |
| 17 | 2.950 | 3.860 | 3.400 | 1.670 | 2.970 | 2.760 | 3.000 | 2,680 | 3.100 | 4.400 | 3.190 |
| 22 | 10.840 | 3.610 | 5.900 | 2.300 | 5.660 | 3.710 | 4,560 | 7.600 | 10,260 | 16,200 | 8.470 |
| 23 | 4.520 | 3.100 | 5.460 | 920 | 3.500 | 2,800 | 3,300 | 4.650 | 4.500 | 7,000 | 4.450 |

[a] The soil was dried at 110° C. for two hours.

If the effect of the various cropping systems in the absence of manure is considered, the various systems rank in the following order in 1914: (1) Timothy, (2) rotation, (3) corn, and (4) wheat. Wheat and corn are about equal, with a marked increase in the rotated and the timothy soils. In the presence of manure the rank is almost the reverse: (1) Corn, (2) wheat, (3) timothy, and (4) rotation. Here, again, wheat and corn are approximately equal, with a marked falling off to timothy and a somewhat less decrease to the rotation.

Just why manure should have the effect of raising the bacterial content of wheat and corn from the lowest to the first rank is not known. On the other hand, just why it should have less effect upon plots with a normally higher count is equally not understood. The peculiar behavior of the rotation plot receiving manure is very striking, the bacterial numbers being only slightly affected. Particularly is this so when we remember that this rotation is composed of corn, oats, wheat, clover, and timothy, since corn, wheat, and timothy bring about conditions which readily respond to manure. It should be remembered that if samples had been taken from rotation plots when some crop other than clover or timothy was growing, the results might have been different. A possible explanation of the lack of effect of manure upon the rotation plots and a less-marked effect upon timothy may lie in the amount of organic matter that these plots themselves return to the soil. The less the quantity of organic matter returned to the soil apparently the more marked is the result from manure.

The results secured from eight analyses of plots 29 and 30 (not given in Table III) indicate that the effect of manure is in part accumulative. Plot 29 had received only 6 applications of manure against 25 for plot 30,

while the average bacterial count for No. 29 was 7,840,000 and for No. 30 was 13,632,000. This accords with Temple's idea (20) that the increase in number is largely due to the fermentable material added and not to the bacteria actually carried in the manure.

FORMATION OF AMMONIA

The results presented in Table IV seem to the writers clearly to establish one of two facts, either that the systems of cropping under study exert no appreciable influence on the ammonia-forming power of this soil type or that the methods used for determining such differences are valueless. Since many investigators have been able to detect marked differences with essentially the same methods, the former conclusion might seem most likely. We would call special attention, however, to the results obtained by Given and Willis (6, 7) and Perotti (15), together with their conclusions regarding the existence of differences and the value of methods in vogue for determining this phenomenon. It is true that during 1913 the plots receiving manure gave slightly higher results than those receiving no such treatment, but it is equally true that the reverse is evident for 1914. Furthermore, there seems to be no correlation between the number of bacteria and the amount of ammonia formed.

It may be argued, in view of the nitrification data given later, that in the plots receiving manure the ammonia formed was transformed into nitrates. But, as previously mentioned, the accumulation of nitrates in seven days' time in the presence of cottonseed meal is practically nil. In fact, the senior writer (5) has demonstrated that under such conditions there is a rapid disappearance of nitrate nitrogen during the first few days. To ascertain this condition, determinations of nitrate nitrogen were made during 1913 which showed, as may be noted, that the above contention was verified. The greatest quantity of nitrate nitrogen recovered after one week's incubation was 3.5 mgm. from plot 10. It is true that there were large differences in the amount of ammonia in the four-week analyses; but if we add the nitrate and ammonia nitrogen, the differences are slight. From the ammonia figures it is also evident that in no case, even after four weeks' incubation, in the absence of calcium carbonate was nitrification limited by the formation of ammonia. This possibly did not hold true in a few cases when calcium carbonate was added.

FORMATION OF NITRATE

In our study of nitrate formation we have obtained the most marked and consistent results of any of our studies. This is graphically shown in figure 1 for the four-week incubated samples without calcium carbonate. Tables IV and V give the tabulated results of experiments conducted on nitrate formation in natural soil from different plots.

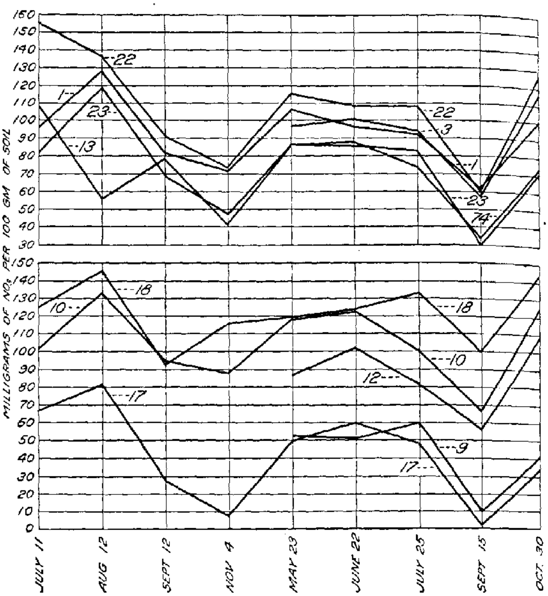


FIG. 1.—Curves of the nitrate formation in soil of fertility plots at Columbia, Mo., in 1913–14, after 28 days' incubation with the addition of 60 mgm. of nitrogen as cottonseed meal, but without the addition of calcium carbonate.

TABLE IV.—*Ammonia and nitrate formation (in milligrams) in soil from different fertility plots at Columbia, Mo., during 7 days' incubation without addition of calcium carbonate*

| Plot No. | 1913 | | | | | | | | | | | | 1914 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nitrate ($NO_3$) recovered. | | | | Nitrogen as ammonia recovered. | | | | Nitrogen as nitrate and ammonia recovered. | | | | Nitrogen as ammonia recovered. | | | | | |
| | Aug. 12. | Sept. 12. | Nov. 4. | Average. | Aug. 12. | Sept. 12. | Nov. 4. | Average. | Aug. 12. | Sept. 12. | Nov. 4. | Average. | May 23. | June 22. | July 25. | Sept. 15. | Oct. 30. | Average. |
| 1 | 3.76 | 8.95 | T.[a] | 4.24 | 39.67 | 27.87 | 31.63 | 33.06 | 40.52 | 29.88 | 31.63 | 34.01 | 27.97 | 26.82 | 24.94 | 20.31 | 24.68 | 24.94 |
| 13 | T.[a] | 2.43 | 0 | .80 | 40.35 | 29.14 | 32.70 | 34.06 | 40.35 | 29.69 | 32.70 | 34.24 | 28.31 | 29.71 | 26.88 | 24.00 | 26.99 | 27.18 |
| 3 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 28.43 | 27.93 | 25.79 | 20.50 | 25.41 | 25.61 |
| 10 | 4.30 | 15.78 | T.[a] | 6.69 | 46.27 | 31.67 | 34.23 | 37.37 | 47.23 | 35.22 | 34.23 | 38.89 | 21.84 | 25.31 | 24.52 | 21.70 | 24.03 | 23.48 |
| 9 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 28.10 | 27.93 | 26.22 | 21.70 | 25.90 | 25.99 |
| 2 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 27.88 | 28.67 | 27.63 | 21.70 | 28.81 | 26.94 |
| 18 | 6.69 | 5.09 | T.[a] | 3.93 | 37.20 | 31.25 | 36.53 | 34.99 | 38.70 | 32.40 | 36.53 | 35.87 | 21.65 | 24.40 | 24.52 | 19.64 | 24.44 | 22.93 |
| 17 | 0 | 1.41 | 0 | .47 | 38.70 | 27.87 | 30.55 | 32.37 | 38.70 | 28.19 | 30.55 | 32.48 | 28.40 | 27.00 | 28.22 | 16.74 | 27.42 | 25.55 |
| 22 | 3.78 | 6.38 | 0 | 3.39 | 42.52 | 25.76 | 31.85 | 33.38 | 43.32 | 27.19 | 31.85 | 34.14 | 26.62 | 28.67 | 25.85 | 23.39 | 25.83 | 26.07 |
| 23 | 0 | 4.06 | 0 | 1.35 | 43.15 | 25.33 | 29.03 | 32.50 | 43.15 | 26.23 | 29.03 | 32.80 | 26.93 | 28.30 | 25.61 | 22.19 | 24.32 | 25.47 |

[a] T.=trace.

TABLE V.—*Ammonia and nitrate formation (in milligrams) in soil from different fertility plots at Columbia, Mo., during 28 days' incubation with and without addition of calcium carbonate*

| Plot No. | 1913 (without calcium carbonate). | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nitrate ($NO_3$) recovered. | | | | | Nitrogen as ammonia recovered. | | | | | Nitrogen as nitrate and ammonia recovered. | | | | |
| | July 11. | Aug. 12. | Sept. 12. | Nov. 4. | Average. | July 11. | Aug. 12. | Sept. 12. | Nov. 4. | Average. | July 11. | Aug. 12. | Sept. 12. | Nov. 4. | Average. |
| 1 | 95.8 | 128.0 | 82.3 | 72.6 | 94.7 | 9.49 | 24.71 | 18.78 | 31.86 | 21.22 | 31.16 | 53.69 | 37.42 | 48.28 | 42.64 |
| 13 | 109.4 | 56.7 | 79.2 | 44.4 | 71.9 | 10.41 | 29.14 | 28.40 | 38.14 | 26.52 | 35.10 | 41.97 | 40.31 | 47.73 | 42.78 |
| 3 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 10 | 101.1 | 133.0 | 95.6 | 88.3 | 104.5 | 8.45 | 22.01 | 19.21 | 22.77 | 18.14 | 31.22 | 51.79 | 41.03 | 42.71 | 41.69 |
| 9 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 2 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 18 | 125.2 | 146.3 | 93.2 | 110.2 | 120.2 | 6.13 | 6.62 | 17.52 | 21.0[illegible] | 13.06 | 34.46 | 39.09 | 38.60 | 47.30 | 40.00 |
| 17 | 67.5 | 82.5 | 28.0 | 7.6 | 46.4 | 13.77 | 41.09 | 40.30 | 51.36 | 36.63 | 29.04 | 59.75 | 46.63 | 53.08 | 47.12 |
| 22 | 155.8 | 127.3 | 91.2 | 71.2 | 114.6 | 7.87 | 22.43 | 14.62 | 31.21 | 19.03 | 33.11 | 53.49 | 35.25 | 17.69 | 34.05 |
| 23 | 81.7 | 110.2 | 69.3 | 47.4 | 79.4 | 11.77 | 33.02 | 29.01 | 39.06 | 28.50 | 30.25 | 59.00 | 45.70 | 50.38 | 46.33 |

| Plot No. | 1914 (without calcium carbonate). | | | | | | 1914 (with calcium carbonate). | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nitrate ($NO_3$) recovered. | | | | | | Nitrate ($NO_3$) recovered. | | | | | |
| | May 23. | June 22. | July 25. | Sept. 15. | Oct. 30. | Average. | May 23. | June 22. | July 25. | Sept. 15. | Oct. 30. | Average. |
| 1 | 105.8 | 97.3 | 92.3 | 62.0 | 100.0 | 91.5 | 141.0 | 133.3 | 144.0 | 146.0 | 175.6 | 147.9 |
| 13 | 80.4 | 87.8 | 75.0 | 34.2 | 74.2 | 71.5 | 161.5 | 153.3 | 138.6 | 58.8 | 163.6 | 135.2 |
| 3 | 97.3 | 100.7 | 94.7 | 58.0 | 116.1 | 93.4 | 150.0 | 156.5 | 138.6 | 92.3 | 165.5 | 140.6 |
| 10 | 120.0 | 124.1 | 100.0 | 67.9 | 124.1 | 107.2 | 164.4 | 147.0 | 150.0 | 120.0 | 165.0 | 149.4 |
| 9 | 52.2 | 51.4 | 60.0 | 10.5 | 41.5 | 43.1 | 139.8 | 125.9 | 129.7 | 50.7 | 171.4 | 123.5 |
| 2 | 87.8 | 102.8 | 81.8 | 56.3 | 109.0 | 87.5 | 144.6 | 153.2 | 141.0 | 73.4 | 158.3 | 134.7 |
| 18 | 120.0 | 124.1 | 143.0 | 100.0 | 144.0 | 124.2 | 150.0 | 156.5 | 150.0 | 100.0 | 156.5 | 142.7 |
| 17 | 50.8 | 60.4 | 48.6 | 21.4 | 34.0 | 39.2 | 163.2 | 130.0 | 107.4 | 20.0 | 72.7 | 102.7 |
| 22 | 110.1 | 109.0 | 109.0 | 61.4 | 126.3 | 104.4 | 159.0 | 151.2 | 141.0 | 100.0 | 163.6 | 143.6 |
| 23 | 85.7 | 85.7 | 82.7 | 30.0 | 71.0 | 71.0 | 147.0 | 153.2 | 150.0 | 100.0 | 167.4 | 143.5 |

Here, again, the marked effect of manure, particularly in the case of corn and wheat, is evident. These two plots rank at the bottom of the series when untreated, but by the addition of manure they are moved from ninth and tenth places to first and second, manure having a much greater influence on these than on any other plots studied. The effect of manure is quite marked on the timothy and rotation plots, though not nearly so great as in the case of corn and wheat. Considering both seasons, we find the percentage of increase caused by the application of manure to be as follows:

| | | 1914 | 1913 |
|---|---|---|---|
| Corn | per cent | 217 | 160 |
| Wheat | per cent | 150 | .... |
| Timothy | per cent | 47 | 44 |
| Rotation | per cent | 28 | 32 |

The increases due to the application of chemicals were as follows: Wheat, 103 per cent; rotation, 30 per cent.

These figures are from the average of those samples receiving no calcium carbonate. Considering the same phenomenon when calcium carbonate was added to the test samples, we find entirely different data: Corn, 39 per cent; wheat, 21 per cent; timothy, no increase; rotation, 9 per cent. When chemicals were added the increase of wheat was 9 per cent; that of the rotation plot, 4 per cent.

The application of calcium carbonate to the soil from different plots in 1914 gives some equally interesting data:

| Plot No. | Cropping system. | Percentage of increase due to calcium carbonate. |
|---|---|---|
| 17 | Continuous corn | 162 |
| 18 | Continuous corn, with the addition of manure | 15 |
| 9 | Continuous wheat | 187 |
| 10 | Continuous wheat, with the addition of manure | 40 |
| 2 | Continuous wheat, with the addition of chemicals | 54 |
| 23 | Continuous timothy | 102 |
| 22 | Continuous timothy, with the addition of manure | 37 |
| 13 | Six-year rotation | 89 |
| 1 | Six-year rotation, with the addition of manure | 61 |
| 3 | Six-year rotation, with the addition of chemicals | 50 |

Calculating from the 1914 tests the percentage increase in the nitrate formation of manured soil with the addition of calcium carbonate over the untreated in the absence of calcium carbonate, we approximate the combined influence of both factors. Estimating the theoretical effect of the two factors by adding the increases resulting from manure alone and from calcium carbonate alone, very different results are obtained.

From these data and those given above it is evident that in only one case of the series studied is it possible to replace entirely the calcium carbonate by manure or the manure by calcium carbonate. However, this may be done to a very large extent. In the case of timothy it is not

possible to replace calcium carbonate with manure. The effect of manure, however, can be entirely eliminated by calcium carbonate. As perhaps would be expected, the percentage increases due to calcium carbonate not replaceable by manure are in an inverse ratio to those of manure not replaceable by calcium carbonate. Such increases seem to be, in general, correlated with the type of crop—that is, those crops naturally depleting the soil of organic matter (corn and wheat) show a large percentage increase from manure not replaceable by calcium carbonate, while those naturally keeping up the organic matter (rotated) show a larger percentage due to calcium carbonate not replaceable by manure.

| Cropping system. | Percentage of increase due to manure and calcium carbonate. | | Difference. |
|---|---|---|---|
| | Actual. | Calculated. | |
| Corn | 264 | 379 | 115 |
| Wheat | 247 | 337 | 90 |
| Timothy | 102 | 149 | 47 |
| Rotation | 107 | 117 | 10 |

It seems evident that in a general way the effect of the two agencies are the same, so far as nitrate formation is concerned. This does not support Temple's contention (20) that the beneficial effect of manure is due to organisms actually brought in with the manure.

| Cropping system. | Percentage of increase due to calcium carbonate in presence of manure or not replaceable by it. | Percentage of increase due to manure in presence of calcium carbonate or not replaceable by it. |
|---|---|---|
| Corn | 15 | 39 |
| Wheat | 40 | 21 |
| Timothy | 37 | 0 |
| Rotation | 61 | 9 |

It is also interesting to note that in the rotation studied either with or without the addition of calcium carbonate, the effect on nitrate formation of the chemicals has been practically identical with that of the manure. In the case of wheat, chemicals have had only about one-half of the beneficial effect that manure has had. As to the effects of the different crops, corn and wheat undoubtedly have a harmful effect in the absence of manure, both ranking very low either with or without calcium carbonate. On the other hand, with the addition of manure they are raised from tenth and ninth places to first and second places, respectively. Timothy and the rotation are approximately equal and very much higher than corn

and wheat. The effect of manure, however, was not very marked on either, being somewhat more pronounced in the case of timothy. When calcium carbonate was added to the test samples the only noticeable effect of the crop is the low position held by corn and wheat in absence of manure.

The fact that the addition of calcium carbonate to samples eliminates to a large extent the very large and unmistakable differences, otherwise detectable, raises the question as to which method probably more accurately represents field conditions. We shall only call attention to the fact that Löhnis and Green (11) vigorously maintain that the addition of calcium carbonate is essential, while Temple (21) has shown that with organic sources of nitrogen vigorous nitrification is possible even in acid soils. Table VI gives the results of an experiment to determine the effect of varying the quantity of nitrogen and calcium carbonate added. This test was run in order to determine the specific quantity of calcium carbonate necessary to insure maximum nitrification and also the correct amount of nitrogen to be added.

TABLE VI.—*Effect on nitrate formation of varying the quantity of calcium carbonate and nitrogen added to soil*

| Plot 17. | | | | | Plot 18. | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Calcium carbonate. | Nitrogen as cottonseed meal. | Nitrate. | Nitrogen as ammonia. | Nitrate. | Calcium carbonate. | Nitrogen as cottonseed meal. | Nitrate. | Nitrogen as ammonia. | Nitrate. |
| *Gm.* | *Mgm.* | *Mgm.* | *Mgm.* | *Mgm.* | *Gm.* | *Mgm.* | *Mgm.* | *Mgm.* | *Mgm.* |
| [a] 0 | 60 | 60 | 60 | 13.7 | [a] 0 | 60 | 104.3 | 60 | 43.2 |
| [a] .05 | 60 | 75.8 | 60 | 23.4 | [a] .05 | 60 | 133.3 | 60 | 66.4 |
| [a] .10 | 60 | 82.7 | 60 | 34.2 | [a] .10 | 60 | 124.7 | 60 | 85.2 |
| [a] .25 | 60 | 94.2 | 60 | 42.0 | [a] .25 | 60 | 135.8 | 60 | 156.5 |
| [a] .50 | 60 | 146.9 | 60 | 55.3 | [a] .50 | 60 | 144.0 | 60 | 218.1 |
| [a] 1.00 | 60 | 141.9 | 60 | 74.0 | [a] 1.00 | 60 | 138.4 | 60 | 232.2 |
| [a] 2.50 | 60 | 144.0 | 60 | 70.8 | [a] 2.50 | 60 | 162.8 | 60 | 244.0 |
| [a] 5.00 | 60 | 144.0 | 60 | 80.0 | [a] 5.00 | 60 | 135.8 | 60 | 244.0 |
| [b] 1 | 0 | 22.3 | 0 | 28.2 | [b] 1 | 0 | 32.5 | 0 | 31.5 |
| [b] 1 | 15 | 56.8 | 15 | 60.0 | [b] 1 | 15 | 58.3 | 15 | 80.0 |
| [b] 1 | 30 | 85.7 | 30 | 75.0 | [b] 1 | 30 | 92.3 | 30 | 108.2 |
| [b] 1 | 60 | 144.0 | 60 | 90.0 | [b] 1 | 60 | 142.8 | 60 | 266.6 |
| [b] 1 | 120 | 193.5 | 120 | 37.2 | [b] 1 | 120 | 244.0 | 120 | 369.0 |
| [b] 1 | 240 | 8.8 | 240 | 7.8 | [b] 1 | 240 | 444.4 | 240 | 232.2 |
| [c] 0 | 0 | 14.0 | 0 | 14.0 | [c] 0 | 0 | 20.0 | 0 | 20.0 |

[a] Nitrogen constant, calcium carbonate varying.
[b] Calcium carbonate constant, nitrogen varying.
[c] Nitrate originally in soil.

The amount of nitrate formed in seven days is so insignificant that we have left the 7-day data out of consideration. It is interesting, however, to note that those plots which rank high for 28 days also rank high for 7 days.

A study of the relative position of the different plots at the various analyses shows clearly that the method used is reliable for detecting

differences, whether the nitrate-forming power is low or high. Table VII gives the relative rank of the different plots at each analysis. Figure 1 illustrates very clearly the low and the high nitrate-forming power at different periods.

TABLE VII.—*Average nitrate formation without addition of calcium carbonate (Table V) of seven fertility plots studied in 1913 and 1914, together with their relative rank at each sampling*

| Plot No. | 1913 | | | | | 1914 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Average nitrate formation. | Rank. | | | | Average nitrate formation. | Rank. | | | | |
| | | July 11. | Aug. 12. | Sept. 12. | Nov. 4. | | May 23. | June 22. | July 25. | Sept. 15. | Oct. 30. |
| | *Mgm.* | | | | | *Mgm.* | | | | | |
| 1 | 94.7 | 5 | 4 | 4 | 4 | 91.5 | 4 | 4 | 4 | 3 | 4 |
| 10 | 104.5 | 4 | 2 | 1 | 2 | 107.2 | 2 | 2 | 3 | 2 | 3 |
| 13 | 71.9 | 3 | 7 | 5 | 6 | 71.5 | 5 | 5 | 6 | 5 | 5 |
| 17 | 46.4 | 7 | 6 | 7 | 7 | 39.2 | 7 | 7 | 7 | 7 | 7 |
| 18 | 120.2 | 2 | 1 | 2 | 1 | 124.2 | 1 | 1 | 1 | 1 | 1 |
| 22 | 114.6 | 1 | 3 | 3 | 3 | 104.4 | 3 | 3 | 2 | 4 | 2 |
| 23 | 79.4 | 6 | 5 | 6 | 5 | 71.0 | 6 | 6 | 5 | 6 | 6 |

Since the addition of calcium carbonate has to such a large extent eliminated the differences in nitrate formation when testing in the soil itself, it is interesting to ascertain whether this can be traced to an elimination of acid conditions. Dr. P. F. Trowbridge, of the Department of Agricultural Chemistry, Missouri Experiment Station, has furnished us with the following data regarding the lime requirements of some of the plots. The figures are in pounds per acre-foot; basis, 3,000,000 pounds per acre: Plot 2, 7,900; plot 13, 7,200; plot 17, 7,900; plot 18, 2,400; plot 22, 2,400; plot 23, 7,200 pounds.

It will be noted that, so far as the crop is concerned, the differences in lime requirements are very slight (plots 13, 17, and 23 or 18 and 22). Manure kept the acidity low (plots 18 and 22). Commercial fertilizers have had no appreciable effect upon acidity (plot 2). Nevertheless, continuous corn soil with a lime requirement not materially different from that of timothy, the rotation, or continuous wheat receiving commercial fertilizers produced only about one-half the amount of nitrates as these soils. It will be shown later that transferring the organisms to a common pabulum containing an abundance of calcium carbonate does not eliminate the large differences. In this connection, as a suggested explanation of one of the effects of lime when added to the soil, we call attention to the work of Schreiner and Reed (16), who have demonstrated the stimulative effect of calcium carbonate upon oxidases. It is not impossible that soil conditions are sometimes such as to permit the accumulation of nitrate-forming oxidases, while other conditions

will not permit similar accumulations. The work of the Bureau of Soils has also demonstrated that calcium carbonate is rather efficient in eliminating the toxic effect of certain decomposition products found in soils, and the beneficial effect might, in part, be due to an action of this nature.

The following data were obtained during 1913 concerning the relative effect of 6 years' application of manure compared with that for 25 years: Plot 29, continuous wheat, received manure for 6 years and produced in one week's incubation an average of 5.35 mgm. of nitrate; in four weeks an average of 116.6 mgm. In the inoculation experiments it produced 15.3 mgm. Plot 30 received manure for 25 years, otherwise similar to No. 29, and produced in one week 6.5 mgm. of nitrate; in four weeks 127 mgm., and in inoculating experiments, 27.5 mgm. This indicates that the shorter period of application has produced almost the same effect as the longer.

## NITRIFYING INOCULATION EXPERIMENTS

The results of the nitrifying inoculation experiments are reported in Table VIII. It should be borne in mind that the 1913 results were obtained under conditions not favorable for nitrate formation, no calcium carbonate having been added. Therefore, too much weight should not be attached to these results. The 1914 results, however, were secured under favorable conditions. It is worthy of note, though, that there is a very close agreement between the relative rank of the plots in the inoculating experiments for both seasons and the nitrate-forming experiments. This is shown in Table IX, together with the relative position of bacterial numbers.

The agreement is not absolute, but it is close enough to indicate the probability of the same factors controlling the nitrate formation in the two instances. This being true, the factors must be biological in nature rather than chemical or physical; otherwise they probably would not be transferred in 20 c. c. of a 2 to 1 soil suspension in sufficient quantities to exercise much influence. There is also exhibited here a close correlation between bacterial numbers and nitrate formation. Since, however, no such correlation can be traced in ammonia formation, there is little reason for believing the two factors connected other than that probably the factors controlling both are the same.

TABLE VIII. *Ammonia and nitrate formation (in milligrams) in soil X when inoculated with soil from different fertility plots during 28 days' incubation.*

| Inoculum plot No. | 1913 Nitrate ($NO_3$) recovered. Aug. 12. | Sept. 12. | Nov. 4. | Dec. 20. | Average. | 1913 Nitrogen as ammonia recovered. Aug. 12. | Sept. 12. | Nov. 4. | Dec. 20. | Average. | 1913 Nitrogen as nitrate and ammonia recovered. Aug. 12. | Sept. 12. | Nov. 4. | Dec. 20. | Average. | 1914 Nitrate ($NO_3$) recovered. May 23. | June 22. | July 25. | Sept. 15. | Oct. 30. | Average. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 34.2 | 31.6 | 0 | 0 | 16.4 | 37.02 | 35.66 | 47.68 | 29.96 | 37.58 | 44.76 | 42.81 | 47.08 | 29.96 | 41.30 | 125.2 | 54.0 | 41.6 | 54.0 | 70.5 | 69.1 |
| 15 | 5.7 | 32.6 | 0 | 0 | 9.6 | 48.81 | 33.25 | 49.10 | 33.01 | 41.06 | 50.10 | 40.39 | 49.19 | 33.01 | 43.17 | 65.4 | 10.3 | 14.3 | 20.3 | 25.7 | 27.2 |
| 3 | | | | | | | | | | | | | | | | 150.0 | 55.3 | 65.2 | 56.2 | 81.8 | 81.7 |
| 10 | 16.2 | 14.7 | 7.0 | 2.0 | 10.0 | 49.63 | 30.57 | 47.03 | 28.72 | 38.99 | 53.29 | 33.89 | 48.01 | 29.17 | 41.25 | 180.0 | 53.3 | 63.1 | 78.2 | 90.1 | 92.9 |
| 9 | | | | | | | | | | | | | | | | 37.1 | 5.0 | 7.5 | 7.4 | 5.2 | 12.4 |
| 2 | | | | | | | | | | | | | | | | 156.6 | 83.0 | 55.5 | 34.8 | 44.4 | 74.9 |
| 18 | 76.7 | 55.1 | 4.7 | 3.3 | 35.0 | 28.40 | 26.71 | 37.93 | 29.96 | 30.70 | 45.76 | 39.20 | 38.09 | 30.09 | 38.67 | 180.0 | 82.7 | 58.2 | 64.3 | 161.1 | 100.3 |
| 17 | 0 | 14.4 | 0 | 0 | 3.6 | 48.36 | 36.02 | 47.68 | 26.28 | 39.58 | 48.35 | 39.27 | 47.68 | 26.28 | 40.38 | 80.0 | 9.8 | 5.3 | Trace. | 6.3 | 20.3 |
| 24 | 23.3 | 38.2 | 0 | 3.2 | 16.2 | 44.38 | 28.02 | 44.64 | 24.45 | 35.37 | 49.65 | 36.66 | 44.61 | 25.17 | 39.03 | 141.7 | 35.0 | 46.1 | 38.5 | 69.6 | 66.2 |
| 23 | 4.0 | 7.0 | 0 | 0 | 2.7 | 52.29 | 32.69 | 46.00 | 19.92 | 37.82 | 53.19 | 34.27 | 46.00 | 19.92 | 38.49 | 93.0 | 10.6 | 33.3 | 20.2 | 8.6 | 33.1 |

TABLE IX.—*Comparison of the ranks in 1913 and 1914 of the bacterial numbers and nitrate-forming and inoculation experiments*

| Plot No. | 1913 | | | 1914 | | |
|---|---|---|---|---|---|---|
| | Bacterial numbers. | Nitrate-forming experiments. | Inoculation experiments. | Bacterial numbers. | Nitrate-forming experiments. | Inoculation experiments. |
| 1.... | 4 | 4 | 2 | 4 | 4 | 3 |
| 13... | 5 | 6 | 5 | 6 | 5 | 6 |
| 10... | 1 | 3 | 4 | 2 | 2 | 2 |
| 18... | 1 | 1 | 1 | 1 | 1 | 1 |
| 17... | 7 | 7 | 6 | 7 | 7 | 7 |
| 22... | 3 | 2 | 3 | 3 | 3 | 4 |
| 23... | 6 | 5 | 7 | 6 | 6 | 5 |

## CROSS-INOCULATION EXPERIMENTS

Unfortunately, time permitted the conducting of only one experiment along the line of cross-inoculation. An effort was made to make this as representative as possible, both by using as the pabulum a mixture of all samples collected from the respective plots during both seasons and by inoculating in duplicate sterile samples, containing 60 mgm. of nitrogen as cottonseed meal from every other plot. In addition, calcium carbonate was added to one of the duplicates. The results are reported in Table X.

Reading horizontally, one obtains figures representing the capacity—using this term in the sense that Stevens and Withers (17) used it—of soil from the different plots to support nitrification both with and without the addition of calcium carbonate. It is noted that all the soils will support vigorous nitrification, provided vigorous nitrifiers are added. If the averages in the last column are examined, it will be noted that when calcium carbonate is added differences can be noted, but they are not so marked as some other results, the highest figure being 99.9 and the lowest 72.7. In the absence of calcium carbonate the figures are not nearly so high, and, though the differences are somewhat more marked, the relative positions are not materially different from those where calcium carbonate was added. Examining any vertical column, we obtain figues representing the ability of the various soils to support the nitrifying flora from any one particular soil; or inversely, the ability of any particular soil to inoculate the others. This also varies materially, indicating that the soil does exercise a marked influence upon nitrification, some floras thriving better in one soil and other floras better in others. Considering the average of the vertical columns, we obtain what may be termed the "relative inoculating ability." Here we obtain our greatest variation, indicating, as previously suggested, that to the flora itself must be ascribed the major differences in nitrification. Here again, the continuously cropped plots, wheat and corn, rank lowest when no manure is added; but manure exercises a greater influence on them than any other in the series. In the case of timothy, manure has not increased its inoculating ability; in fact, the reverse is true, while with the rotated plot the effect has not been nearly so great as with corn and wheat.

TABLE X. *Results of cross-inoculation soil experiments expressed in milligrams of nitrate per 100 gm. of soil*

| Plot No. | Nitrate ($NO_3$), with addition of calcium carbonate. | | | | | | | | | | | Nitrate ($NO_3$), without addition of calcium carbonate. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Inoculum..... | 1 | 2 | 3 | 9 | 10 | 13 | 17 | 18 | 22 | 23 | Average. | 1 | 2 | 3 | 9 | 10 | 13 | 17 | 18 | 22 | 23 | Average. |
| 1............. | 72.0 | 108.0 | 108.0 | 0 | 180.0 | 86.4 | 24.0 | 112.5 | 36.6 | 83.7 | 81.1 | 36.0 | 33.5 | 8.3 | 0 | 45.9 | 12.2 | 0 | 35.4 | 0 | 12.6 | 18.4 |
| 13............. | 116.1 | 100.0 | ........ | 0 | 109.0 | 75.0 | 26.1 | 116.1 | 85.7 | 92.3 | 80.0 | 21.0 | 31.3 | 14.6 | 0 | 56.2 | 33.2 | 0.2 | 25.7 | 6.6 | 22.5 | 21.7 |
| 3............. | 124.1 | 63.5 | 56.8 | 0 | 171.4 | 83.8 | 9.0 | 112.5 | 25.7 | 80.0 | 72.7 | 31.7 | 32.7 | 7.6 | 0 | 69.6 | 15.0 | 0 | 24.8 | 0 | 9.2 | 19.1 |
| 10............. | 150.0 | ........ | 128.5 | 4.0 | 180.0 | 94.7 | 17.4 | 112.5 | 109.0 | 69.2 | 96.2 | 60.0 | ...... | 12.8 | 0 | 40.0 | 29.1 | 4.1 | 33.7 | 7.8 | 20.0 | 20.7 |
| 9............. | 120.0 | 90.0 | 56.8 | 0 | 163.6 | 86.4 | 30.8 | 128.5 | 38.5 | 62.0 | 77.7 | 27.1 | 25.7 | 5.1 | 0 | 30.8 | 18.0 | 0 | 27.3 | 0 | 7.8 | 14.2 |
| 2............. | 128.5 | 138.4 | 94.7 | 0 | 133.3 | 43.2 | 6.0 | 85.7 | 38.5 | 85.7 | 75.4 | 34.8 | 46.9 | 8.1 | 0 | 31.3 | 16.3 | 0 | 28.8 | 0 | 11.6 | 17.8 |
| 17............. | 94.7 | 97.3 | 105.8 | 0 | 133.4 | 87.8 | 30.8 | 81.8 | 46.9 | 54.5 | 73.3 | 12.0 | 15.6 | 3.9 | 0 | 27.7 | 23.3 | 5.0 | 23.2 | 0 | 17.1 | 12.8 |
| 18............. | 144.0 | 130.0 | 100.0 | 0 | 144.0 | 87.8 | 42.3 | 128.5 | 60.0 | 94.7 | 92.1 | ...... | 65.4 | 15.6 | 0 | 75.0 | 42.3 | 13.3 | 63.1 | 4.8 | 27.6 | 34.1 |
| 22............. | 128.5 | 133.3 | 109.0 | 0 | 150.0 | 150.0 | 46.9 | 144.0 | 21.2 | 94.7 | 97.8 | 25.7 | 56.8 | 17.1 | 0 | 76.5 | 45.9 | 9.7 | 70.5 | 5.3 | 30.4 | 34.4 |
| 23............. | 156.5 | 86.4 | 144.0 | 0 | 180.0 | 138.4 | 45.0 | 150.0 | 52.6 | 45.9 | 100.8 | 43.2 | 25.4 | 12.4 | 0 | 70.5 | 50.2 | 7.2 | 54.0 | 0 | 28.2 | 29.7 |
| Average. | 137.1 | 104.1 | 100.4 | 0.4 | 154.4 | 93.3 | 27.8 | 117.2 | 51.5 | 76.3 | ........ | 32.4 | 37.0 | 10.5 | 0 | 52.9 | 28.5 | 4.5 | 39.3 | 2.2 | 18.7 | ...... |

The nitrifying capacity averages in the last column of Table X place timothy at the top of the list, manure in this case having no effect. Next in order are wheat and corn, where manure is added. Manure had no increasing effect on the rotated plot. The continuously cropped plots without manure, the rotated plots, and the plots receiving chemicals vary little. Manure here exercises little or no influence on those crops not depleting the soil of organic matter.

It is evident that the nitrifying floras of plots 9, 17, and 22, the first two especially, were extremely weak. Though these plots possessed at this time good nitrate-forming floras, they were not able to overcome the adverse effects experienced in transferring them to soils slightly less favorable but in which a more vigorous or differently constituted flora thrived.

## NITRATE NITROGEN UNDER FIELD CONDITIONS

In Table XI is given the quantity of nitrogen as nitrate per 100 gm. of soil when the soil was collected from the field, but nothing particularly marked is to be noted from the results given. As would be expected, this quantity varies considerably; but since the demands made upon the various plots by the growing crop differ widely, little information is furnished regarding the rate of formation. After the wheat is harvested the nitrate content of wheat plots increases rapidly. Wheat was cut on June 20, 1913, and on June 28, 1914. Some accumulation is evident even before harvest. When wheat is growing rapidly no nitrates are present. The accumulation is much more marked in the presence of manure and chemicals than in their absence. There is an abundance of nitrate nitrogen under corn even when it is making its most rapid growth. During 1913 the water content fell so low in July and August that no nitrates could be formed; with rain coming in October, however, the nitrate content rose rapidly. In all cases where a comparison of the same crop in the presence and absence of manure is possible the nitrate content of the manure plot is materially higher than that of the unmanured.

TABLE XI.—*Quantity (in milligrams per 100 grams of soil) of nitrate in soil when sampled from fertility plots at Columbia, Mo., in 1913 and 1914* [a]

| Plot No. | Quantity of nitrate ($NO_3$). | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1913 | | | | | | 1914 | | | | | |
| | July 11. | Aug. 12. | Sept. 12. | Nov. 4. | Dec. 20. | Average. | May 23. | June 22. | July 25. | Sept. 15. | Oct. 30. | Average. |
| 1 | 3.91 | 2.06 | 10.66 | 7.71 | 2.72 | 5.41 | 1.94 | 3.20 | 4.00 | 1.50 | 1.60 | 2.45 |
| 13 | 1.43 | .75 | 1.07 | 1.90 | 1.25 | 1.28 | 2.10 | 1.13 | 2.10 | 0.75 | Trace. | 1.22 |
| 3 | ..... | ..... | ..... | ..... | ..... | ..... | 0 | 1.27 | 2.00 | 1.20 | 1.50 | 1.19 |
| 10 | 6.40 | 8.78 | 18.00 | 11.08 | Trace. | 8.85 | 1.97 | 3.60 | 8.50 | 6.40 | 5.00 | 5.09 |
| 9 | ..... | ..... | ..... | ..... | ..... | ..... | 0 | 1.71 | 5.50 | 4.50 | 3.00 | 2.94 |
| 2 | ..... | ..... | ..... | ..... | ..... | ..... | 0 | 2.70 | 4.80 | 4.50 | 7.50 | 3.90 |
| 18 | 4.48 | .75 | 4.46 | 5.67 | 3.57 | 3.79 | 2.40 | 6.00 | 12.20 | 7.20 | 2.50 | 6.06 |
| 17 | 2.48 | .66 | .53 | 2.10 | 3.06 | 1.76 | 3.10 | 6.12 | 6.43 | 1.70 | 1.00 | 3.85 |
| 22 | 1.94 | 1.91 | 6.50 | 1.90 | Trace. | 2.45 | 2.10 | 1.80 | 3.60 | 5.40 | 3.10 | 3.20 |
| 23 | 1.38 | 1.28 | 2.73 | Trace. | Trace. | 1.08 | 0 | 0 | 1.80 | 2.90 | 1.20 | 1.18 |

[a] Soil was dried at 110° C. for two hours.

## SUMMARY

(1) The agricultural methods practiced upon the plots under study have brought about marked differences in the number of organisms contained in the soil, at least those capable of developing under our experimental conditions. The soil under continuous corn and wheat contains, in the absence of any additions of fertilizers or manure, relatively low numbers of bacteria. In the presence of manure, continuous corn and wheat soil contain relatively high numbers, manure having a much more marked effect upon numbers here than under the other crops studied.

(2) The agricultural practices under study have, so far as we can detect without experimental methods, produced no appreciable effect upon the ability of the soil and its organic life to liberate ammonia from cottonseed meal.

(3) The ability of the soil complex to oxidize ammonia nitrogen to nitrate nitrogen has been materially altered by the methods under study. This we believe to be due in part to physical and chemical changes in the soil and in part to biological changes. Continuous corn and wheat with no additions of manure or chemicals have brought about a relative low oxidizing power in the soil complex. The addition of manure materially raises the oxidizing power, especially under continuous corn and wheat. The addition of commercial fertilizer brings about a condition similar to that of manure, though perhaps less marked.

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